UNIVERSITY OF CAPECOAST

EVALUATION OF THE EFFECTIVENESS OF SULFADOXINE-

PYRIMETHAMINE (SP) AS ANTIMALARIAL PROPHYLAXIS IN

PREGNANT WOMEN IN SELECTED HEALTH FACILITIES IN

CENTRAL REGION

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BY

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THESIS SUBMITTED TO THE DEPARTMENT OF HUMAN BIOLOGY OF THE SCHOOL OF BIOLOGICAL SCIENCES, FACULTY OF SCIENCE, UNIVERSITY OF CAPE COAST IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF MASTER OF PHILOSOPHY DEGREE IN PARASITOLOGY

APRIL, 2011

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original work and that
no part of it has been presented for another degree in this university or
elsewhere.

Candidate's Name: DANNY FLINT YEBOAH

Signature……………………   Date………………………….

Supervisor's Declaration

We hereby declare that the preparation and presentation of the thesis were
supervised in accordance with the guidelines on supervision of thesis laid
down by the University of Cape Coast.

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ABSTRACT

The use of sulfadoxinepyrimethaine (SP) as an intermittent preventive treatment (IPT) against malaria during pregnancy has become a policy in most sub-Sahara African countries but crucially depends on the efficacy of SP. This study sets out to evaluate the effectiveness of the SP given to the pregnant women in the selected health facilities in the Central region of Ghana to prevent maternal malaria in the pregnant women. A cross-sectional study was carried out to evaluate the effectiveness of SP in clearing malaria parasites in 543 pregnant women recruited from 7 selected health centres in Central Region of Ghana. To determine the quality of SP, high performance liquid chromatography (HPLC) was used to assay the SP in samples of the tablets given to the pregnant women. The tablets were taken through dissolution test. The parasite density of *Plasmodium falciparum* was determined from the peripheral blood of the pregnant women using microscopy. Haemoglobin levels as well as ABO blood types were determined. The pregnant women did not receive IPT-SP because of either unavailability of the drug or they were not due to take SP were 44.0%. Malaria infection was recorded in 11.2% of pregnant women who had a history of SP consumption. Low haemoglobin level was recorded in 73.5% of the pregnant women. Pregnant women with blood group O had the highest frequency of 55.2% of the population. SP was found to be sub-standard because it failed the dissolution test. IPT-SP is ineffective in preventing malaria infection. Manufacturing practice of SP should be improved as well as in the stocking of SP tablets at the health centres to make it readily available to the pregnant women.

ACKNOWLEDGEMENT

DEDICATION

To my mum Elizabeth Dufie-Boakye and aunt Grace Gifty Boakye
and her family.

TABLE OF CONTENTS

CHAPTER TWO: LITERATURE REVIEW

CHAPTER FIVE: DISCUSSION

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

APPENDICES

CHAPTER ONE

INTRODUCTION

Malaria

Malaria is an infectious life threatening parasitic disease. The disease is caused by a protozoan parasite called *Plasmodium* belonging to the subphylum *Apicomplexa* which infects erythrocytes. The parasite is transmitted through the bites of the blood-feeding female anopheline mosquito (White, 2009). There are approximately 156 named species of *Plasmodium* which infect various species of vertebrates. There *Plasmodium* species of medical interest are*P. vivax, P. ovale, P. malariae* and *P. falciparum*. There have been few reported cases of *P.knowlesi* infection in South –East Asia (WHO, 2010).

The risk of malaria infection varies with the infecting species of *Plasmodium* and with the individual's prior health and immune status. Malaria is characterized by frequent fever and chills, along with headaches, vomiting, and diarrhoea. Infection from*P. falciparum* can cause cerebral malaria; a frequently fatal condition involving the brain and central nervous system. Severe disease episodes, that is, cerebral malaria have been shown to cause severe long term physical and neurological disability. Those who survive cerebral malaria may experience lasting brain damage (Gollin and Zimmermann, 2007). Malaria hasbeen associated with maternal anaemia during pregnancy, low birth weight for babies, and also a major cause of childhood anaemia (Gollin and Zimmermann, 2007).

Malaria begins when the female anopheline mosquito injects plasmodial sporozoites into the host at the time of feeding. The sporozoites enter the circulation and bore into the hepatocytes and undergo asexual reproduction. The hepatic schizonts rupture to release merozoites into the bloodstream. The merozoites invade erythrocytes rapidly. The parasites multiply within the red blood cell as they consume the content of the erythrocyte. The erythrocyte eventually becomes full of merozoites. It then ruptures, so that the merozoites are released. The released merozoites rapidly re-invade other erythrocytes and start a new asexual cycle. Sexual forms of the parasite (gametocytes) develop after a series of asexual cycles. Following ingestion in the blood meal of a biting anopheline mosquito, the male and female gametocytes further develop, undergo fusion and multiply to form sporozoites. The sporozoites then migrate to the salivary glands of the mosquito from where they could be inoculated into the next human host during feeding (White, 2009).

Environmental change and malaria

The prevalence of the malaria disease varies across the globe, largely due to differences in the human exposure to Anopheles mosquito bites, the genetic constitution of the host and the immune status (Weatherall et al., 2002). Malaria transmission is also affected by climate and geography (Fontenille et al., 1997), increased drug resistance and the lack of adequate vector control measures (Nkuo-Akenji et al., 2006). Mosquitoes are not found in areas of intense cold or in deserts (Sachs and Malaney, 2002). Human exposures are also reduced in areas where mosquitoes spend winter months as

eggs or in dormant stages of their life cycle. Exposures may also be reduced in areas where people spend significant fractions of their time indoors in enclosed or screened buildings, or where people are dressed in ways that will reduce exposure (Gollin and Zimmermann, 2007). Malaria transmission does not occur at temperatures below 16 º C or above 33 º C, and at altitudes >2000 m because development in the mosquito (sporogony) cannot take place (Gillies, 1988). The optimum conditions for transmission are high humidity and an ambient temperature between 20 º C and 30 º C. Although rainfall provides breeding sites for mosquitoes, excessive rainfall may wash away mosquito larvae and pupae (Gillies, 1988).

High incidence of malaria coincides with increased mosquito abundance (Cook and Zumla, 2009). In some areas, parasite rates (i.e. the proportion of people with positive blood smears) are relatively constant throughout the year, but the majority of cases still do occur during the wet season. In Europe, before malaria eradication, *P. falciparum* malaria was common in spring and in late summer and autumn, and was termed 'aestivo-autumnal malaria' (Cook and Zumla, 2009).The intensity of transmission can change. For example in Africa, the sub-Sahelian drought has reduced rainfall and mosquito transmission in countries such as Senegal and the Gambia. In the 1960s, transmission was not intense, and severe disease was rare in children over 3 years of age (Brewster and Greenwood, 1993; McGregor, 1965). However in recent times, changes in agricultural practice and decreased sanitary conditions in urban areas have promoted malaria transmission (Messina et al., 2011). Urban malaria is becoming an increasing problem in many countries (Cook and Zumla, 2009).

Malaria epidemiology

Malaria epidemics do not generally occur in high transmission areas because the populations of these areas develop partial immunity to the disease (WHO, 2004). In low transmission settings, malaria can behave as an epidemic disease carrying a high mortality. Epidemics are caused by three main factors: human, vector and parasite related factors. Human related factors include relative immunity, migration and vulnerability due to other factors such as increasing malnutrition, HIV-positive etc.(WHO, 2004). Vector related factors also include increased breeding possibilities, the introduction of new and more efficient vectors, vector control broken down and insecticide resistance (WHO, 2004). Resistance to antimalarial drugs is the main parasite factor (WHO, 2004). Epidemics have occurred in North India, Sri Lanka, South-east Asia, Ethiopia, Madagascar, Brazil (when the formidable African vector *Anopheles gambiae* was inadvertently imported from Africa in the 1930s) and more recently in Burundi and KwaZulu Natal where drug resistance was also a contributory factor (Cook and Zumla, 2009).

Increasing international air travel and worsening antimalarial drug resistance have led to an increase in imported cases of malaria in tourists, travellers and immigrants. Imported malaria mostly occurs in tourists and migrants travelling to their origin countries to visit friends and relatives (Castelli, Odolini, Autino, Foca and Russo, 2010). The incidence of malaria has risen markedly in several African countries, India, and Bangladesh over recent decades (Cook and Zumla, 2009). Imported malaria is often misdiagnosed, leading to delays in treatment and severe presentations of *falciparum* malaria are not uncommon. Malaria may also be transmitted by

blood transfusion, transplantation, or through needle-sharing among intravenous drug addicts (Gillespie and Pearson, 2001, Harinasatu and Bunnang, 1988).

Global distribution of malaria

According to United Nations Children's Fund (UNICEF) and Roll Back Malaria (RBM) (2007) an estimated 3 billion people, almost half the world's population, live in areas where malaria transmission occurs. Malaria is endemic in 106 countries for 2010. Malaria is one of the most important parasitic diseases affecting sub-Saharan Africa (Enato and Okhamafe, 2005).There were 216 million cases of malaria in 2010 and 81% of these were in sub-Saharan Africa (WHO, 2011). An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). An estimated 655 000 persons died of malaria in 2010 (WHO, 2011). Children under 5 years of age formed 86%of thevictims, and 91% of malaria deaths occurred in sub-Sahara Africa (WHO, 2011). About 60%, or 390 000 of malaria deaths occurred in six countries Nigeria, the Democratic Republic of Congo, Burkina Faso, Mozambique, Cote d'Ivoire and Mali (WHO, 2011).

Over 50 million pregnant women a year are exposed to malaria resulting in 2,500 – 10,000 maternal deaths annually (Morley and Taylor-Robinson, 2012), at least 60 percent of them in Africa. In sub-Sahara Africa, 25 million pregnant women are at risk of *Plasmodium falciparum* infection every year, and one in four women has evidence of placental infection at the time of delivery (Desai et al., 2007). In high transmission areas, malaria is associated with maternal anaemia (potentially responsible for maternal death

when severe) and low birth weight due to both prematurity and intrauterine growth retardation (Briand, Cottrell, Massougbodji and Cot, 2007).

Economic impact of malaria

Malaria causes morbidity and mortality with obvious economic consequences. The direct individual economic impacts of the disease include the value of lives lost, the value of time lost to sickness, care-giving time spent by other family members (Uguru, Onwujejwe, Uzochukwu, Igiliegbe and Eze, 2009) and the expenditures on medical care, treatment, and prevention. Direct social costs include government expenditures on malaria control and prevention. The indirect costs may be greater still. These include changes in human settlement and labour patterns induced by disease for example, changes in the locations where people live or farm. Indirect costs also include the consequences of the disease on fertility, demography, and human capital investment; and potentially on managerial quality and technology adoption. For example, skilled managers may prefer not to work in malarial regions, resulting in reduced productivity levels (Sachs and Malaney, 2002).

Malaria slows economic growth in poor countries. Malaria prevalence is negatively related to growth of per capita income. Sub-Saharan African countries experience a reduction in income growth of 0.55 percent annually because of malaria (Gollin and Zimmermann, 2007). Countries with intensive malaria experience a reduction in per capita income growth of 1.3 percent annually. All things being equal, a country experiencing endemic malaria would have its long-term level of per capita reduced by one-third, compared with the same country in the absence of malaria (Gallup and Sachs, 2001).

Global spending on malaria prevention and control is around $100-200 million annually (Gollin and Zimmermann, 2007).

Malaria situation in Ghana

Malaria is the leading cause of morbidity in Ghana, accounting for about 37.5 percent of Out Patient Department (OPD) attendance (Duanor et al*.,* 2010). In Ghana, malaria accounts for approximately 44 percent of reported outpatient cases and an estimated percent of mortality in children under 5 years of age at primary health care facilities (Gyapong et al., 2009). Severe malaria in children under five years recorded in 2003 was about 78,392 but the number dropped to 61,462 in 2009, while severe malaria in pregnant women in the same year was at 7,252 and dropped to 4,619 in 2009 (Duanor et al., 2010). The number OPD deaths recorded due to malaria in 2009 was 3,900 (Duanor et al., 2010). Malaria illness cost the country an estimated 21.965 million Ghana cedis translating into 112,832 cedis per household annually. This figure is equivalent to 9.74 percent of per capita government expenditure on health and an average cost per case to the Ministry of Health and Ghana Health Service is estimated at 24,571.53 cedis (Baafi and Kodzitse, 2010). Malaria is endemic in Ghana and among pregnant women. It accounts for 28.1 percent of Out Patient Department(OPD) attendance, 13.7 percent of hospital admissions and 9.0 percent of maternal deaths (Ahiable, 2009).

Malaria treatment and control

Vector control is the primary public health intervention for reducing malaria transmission (Reddy et al., 2011). In high transmission areas, it is able to reduce child mortality rates and the prevalence of severe anaemia. Two effective vector control methods include:

1. Sleeping in long lasting insecticide impregnated nets. This provides a sleeping individual a physical barrier against the bite of an infected mosquito. In addition the net kills or repels mosquitoes that rest on it. However, because of its high cost, many people are unable to afford it. In hot climates, inhabitants may sleep outside, where there is no place to hang a net (Heggenhougen, Hackethal, and Vivek, 2003).

2. Indoor spraying with residual insecticides, which involves applying a long lasting insecticide to the inside of house and other structures to kill mosquitoes resting on interior walls. The challenge with this intervention is that, there is an increasing mosquito resistance to insecticides, including DDT and pyrethroids, and lack of alternative, cost effective and safe insecticides particularly in Africa (WHO, 2010).

Prompt and effective treatment of malaria within 24 hours of the onset of symptoms is necessary to prevent life threatening complications. The challenges to providing prompt and effective treatment for malaria in Africa are: firstly, the majority of malaria cases are not seen within the formal health sector. Secondly, case management has relied largely on antimalarials mainly chloroquine and sulfadoxine-pyrimethamine (SP), which though inexpensive and widely available and are eliminated slowly from the body (White, 2004), are no longer very effective. The extensive deployment of these antimalarial

drugs, in the past fifty years has provided a tremendous selection pressure on human malaria parasites to evolve mechanisms of resistance. Chloroquine has become ineffective for the treatment of *P. falciparum* malaria in many parts of the world due to development of resistance by the parasite (Shujatullah, Khan, Khatoon, Khan and Ashfaq, 2012). Resistance to SP is also widespread and has developed much more rapidly (White, 2004). Resistance to mefloquine is confined to only those areas where it has been widely used (Nosten, 2000). The use of substandard antimalarial drugs has contributed to resistance of the malaria parasite to the drug. Substandard drugs are not only ineffective and sometimes hazardous, they also hasten the development of strains of the malaria parasite that are resistant to the active ingredients (Chinnock, 2010).

The development of a malaria vaccine is still in progress in the laboratories, and no potent vaccine is available (Dhanawat, Das, Nagarwal and Pandit, 2010) for malaria that provides a high level of protection. Preventive drugs must therefore be taken continuously to reduce the risk of infection.

Currently, the most reliable effective antimalarial drugs are artemisinin-based combination therapies (ACTs)(Malisa and Kiriba, 2012). ACTs treat malaria using a combination of two drugs, one of which must be a derivative of artemisinin − the most effective antimalarial ever discovered. It acts very quickly. The most widely used derivatives are artesunate and artemether. For use as a first-line drug for uncomplicated malaria, one of these two has to be combined with another longer-acting antimalarial, one known to have a low resistance in the geographical region in question. Only four ACTs are currently in use and they are; artesunate plus sulphadoxine/pyrimethamine,

artesunate plus amodiaquine, artesunate plus mefloquine and artemether plus lumefantrine (Hook, 2005).

World Health Organization (WHO) policy for malaria prevention and control during pregnancy in areas of stable transmission emphasizes a preventive package of intermittent preventive treatment (IPT) and insecticide-treated bed nets (ITNs) and effective case management of malaria illness and anemia. WHO has recommended that all pregnant women in areas of stable malaria transmission should receive at least 2 doses of IPT after quickening. The most effective drug for IPT is sulfadoxine-pyrimethamine (SP) because of its safety for use during pregnancy, effectiveness in reproductive-age women, and feasibility for use in programmes, as it can be delivered as a single-dose treatment under observation by the health worker (WHO, 2004).

Diagnosis of malaria

Clinical diagnosis of malaria is based on the patients' signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhoea, nausea, vomiting, anorexia, and pruritus (Looareesuwan, 1999). A clinical diagnosis of malaria is still challenging because of the nonspecific nature of the signs and symptoms, which overlap considerably with other common, as well as potentially life-threatening diseases, for example, common viral or bacterial infections, and other febrile illnesses. The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for

patients with non-malarial fevers in endemic areas (Mwangi, Mohammed, Dayo, Snow and Marsh, 2005). The accuracy of malaria diagnosis can therefore be greatly enhanced by combining clinical and parasite based findings (Kyabayinze, Tibenderana, Odong, Rwakimari and Counihan, 2008).

In the laboratory, malaria is diagnosed using different techniques, e.g. conventional microscopic diagnosis by staining thin and thick peripheral blood smears (Ngasala et al., 2008). Other concentration techniques, like quantitative buffy coat method (Bhandari, Raghuveer, Rajeev and Bhandari, 2008), rapid diagnostic test e.g. OptiMAL (Tagbor, Bruce, Browne, Greenwood and Chandramohan, 2008), Paracheck (Harvey et al., 2008) and molecular diagnostic methods, such as polymerase chain reaction (Holland and Kiechle, 2005) may also be used for malaria diagnosis.

Statement of the problem

In areas of full implementation of IPT in pregnancy (IPTp), increasing resistance to SP is a growing challenge. Already in parts of Africa and Southeast Asia, the effectiveness of SP in IPT in pregnancy is being threatened by increasing levels of resistance to SP (Adedeji et al., 2010).

Hypothesis

IPT in pregnancy with SP is incapable of preventing malaria infection.

Rational

According to Mockenhaupt et al., (2007), at the Agogo hospital in Ghana, clinical and parasitological parameters improved following the

implementation of intermittent preventive treatment in pregnancy using SP. However, the prevalence of malaria is now considerable. The usefulness of SP for IPT in countries facing moderate to high levels of SP resistance need to be evaluated (WHO, 2004). SP treatment failure occurred in pregnant women in central Ghana during 2003 – 2004 (Tagbor, Bruce and Ord, 2007). Treatment failure might be as a result of the administration of sub-standard standard drugs or the development of resistance of the parasite to the drug. A study to determine the efficacy of SP given as IPT is therefore in the right direction. The outcome of the study would contribute towards malaria policy formulation.

Goal and objectives

The goal of this study is to determine whether IPT with SP is able to eliminate malaria parasites from the peripheral blood of pregnant women.

The specific objectives are

1. To determine malaria infection in pregnant women who have taken SP.

2. To compare the haemoglobin levels of malaria infected pregnant women with those of the uninfected pregnant women.

3. To determine the parity level that is more susceptible to malaria infection.

4. To determine the relationship between the seasons and parasite densities in pregnant women.

5. To determine the predominant and the most malaria susceptible blood group type among the pregnant women.

6. To ascertain the effect of the level of maternal education on the parity level among the pregnant women.

7. To determine the quality of SP given to the pregnant women at the hospitals and two other SP drugs found at pharmacies and chemical shops.

CHAPTER TWO

LITERATURE REVIEW

Malaria cases among pregnant women

Malaria infection during pregnancy is a major public health problem in tropical and subtropical regions of the world. In most endemic areas of Africa, pregnant women are the main adult risk group for malaria. Annually, approximately 25 million African women become pregnant and are at risk of *Plasmodium falciparum* malaria infection during pregnancy (Steketee et al.*,* 2001). The adverse effects of malaria during pregnancy are very grave. Although frequently asymptomatic, consequences of *Plasmodium falciparum* in pregnancy comprise maternal anaemia, abortion, stillbirth, intrauterine growth retardation, low birth weight, preterm delivery, and up to 200,000 attributable infant deaths per year (Brabin, 1983; Parise, Ayisi and Nahlen, 1998; Shulman et al., 1999; Sullivan et al., 1999; Steketee et al., 2001 and van Geertruyden, Thomas, Erhart and D'Alessandro, 2004). These effects are caused by the presence of malaria parasites in the placenta (Agomo, Oyibo and Odukoya-Maije, 2011). These parasites at the placental sites lead to general placental thickening thereby hindering the passage of nutrients and oxygen from mother to the foetus (Alvarez, Al-Khan and Apuzzio, 2005). The use of the antimalarial drugs given in treatment doses clears the placenta of the parasites, allowing the free passage of nutrients and oxygen to the foetus. This minimizes the chances that a foetus will suffer the effects of malaria. It also

reduces the chances that a mother will end up with maternal anaemia (GHS, NMCP, JHPIEGO, MSH and Global Fund, 2005). Malaria contributed to an estimated 400,000 cases of anaemia among pregnant African women in 1995 (Guyatt and Snow 2001) and indirectly caused about 100,000 infant death through low birthweight (Guyatt and Snow 2001).

Intermittent preventive treatment

For many years WHO recommended that pregnant women in malaria endemic areas should receive an initial antimalarial treatment dose on their first contact with antenatal services, followed by weekly chemoprophylaxis (given at less than therapeutic dose) with an effective and safe antimalarial drug (WHO, 1986). In most countries in Africa, chloroquine has been the drug of choice. However, the emergence and spread of chloroquine resistant falciparum malaria, poor patient compliance with multiple doses, and a high incidence of chloroquine induced pruritis have limited the effectiveness and its importation into countries like Cameroon has been officially stopped in 2002 (Whegang et al., 2010).The first African country to change the national treatment policy from chloroquine to sulphadoxine-pyrimethamine was Malawi in 1993, followed 4 years later by Kenya, South Africa (other than KwaZulu-Natal), and Botswana (Bloland et al., 1998). Sulfadoxine-pyrimethamine (IPT-SP) is more cost-effective than chloroquine chemoprophylaxis in primigravidae in an area of moderate to high malaria transmission, largely because of lower costs and higher compliance with SP (Goodman, Coleman and Mills, 2001). In a further cost-effectiveness analysis, three different regimens were compared with febrile case management using

SP for a hypothetical cohort of 10,000 pregnant women in Kenya. The results showed the two-dose SP regimen to be the least expensive strategy for preventing low birth weight (Wolfe, 2001).

In 2000, the World Health Organization Expert Committee on Malaria recommended that intermittent preventive treatment (IPT) with an effective and safe, preferably one dose antimalarial drug should be made available as a routine part of antenatal care to womenin their first and second pregnancies in highly endemic areas (WHO, 2000). IPT involves the periodic presumptive administration (irrespective of parasitaemia) of full curative treatment doses of an effective antimalarial drug at predefined intervals to all pregnant women. The administration of the antimalarial drug commences after 16 weeks pregnancy or after quickening as part of routine antenatal care. Intermittent preventive treatment has proven efficacious in reducing the burden of malaria in pregnancy (Valley, Valley, Changalucha, Greenwood and Chandramohan, 2007). WHO recommends a schedule of 4 antenatal clinic visits, with three visits after quickening (WHO, 2004). The delivery of IPT with each scheduled visit after quickening ensures that a high proportion of women receive at least 2 doses. IPT-SP doses should not be given more frequently than monthly. Currently, sulfadoxine-pyrimethamine (SP) is the WHO recommended drug for prevention of malaria during pregnancy where transmission of *Plasmodium falciparum* malaria is stable and where resistance to SP is low (Briand, Dennoeud, Massougbodji and Cot, 2008) for the following reasons;

a) Effectiveness: SP is the single dose antimalarial with the best overall effectiveness for prevention of malaria in pregnancy in areas of Africa with

stabletransmission of *Plasmodiumfalciparum* malaria and also where resistance to SP is low.

b) Efficacy: Very good in clearing susceptible placental parasites.

c) Safety: No significant side effect when used appropriately in pregnancy has been observed.

d) Acceptance: Demonstrated high levels of IPT acceptance by pregnant women.

e) Compliance: Good because drug is delivered as a single dose treatment under observation by health worker and thereby minimizing compliance problems.

The standard SP contains 500mg sulfadoxine and 25mg pyrimethamine (WHO, 2010). SP given at therapeutic dose – is the single-dose antimalarial with the best overall effectiveness for prevention of malaria in pregnancy in areas with high transmission, and low resistance to SP. In areas of stable malaria transmission, non-pregnant adults have high levels of immunity to malaria and usually do not become severely ill with infection. However, pregnant women, especially primigravidas have increased susceptibility to malaria. One dose of intermittent preventive treatment with the drug combination sulfadoxine-pyrimethamine during the first 26 weeks of pregnancy has been shown to decrease a woman's risk of malaria by 85% and anaemia by 50% (Czechowicz, Maldonado and Benavente, 2006)

Some precautionary measures taken with regards to the administration of SP includes, SP

a) is not given to a pregnant woman in the first trimester (less than 13 weeks of gestation).

b) is not given to a pregnant woman who has received recent treatment with SP (less than one month ago).

c) is not given to a pregnant woman who is allergic to sulphur drugs.

d) should not be given to a pregnant woman who is taking co-trimoxazole to treat other infections (GHS, NMCP, JHPIEGO, MSH and Global Fund, 2005).

IPT with SP in pregnancy in some African countries

In 2000 the Roll Back Malaria (RBM) movement set a target of providing for at least 60% of pregnant women with an insecticide treated net (ITN) and at least two courses of IPT by the year 2010 (WHO, 2000). Owing to WHO's recommendations of IPT as a protection for pregnant women against malaria infection in malaria endemic areas, many African countries adopted IPT as a national policy.

In April 2001, the Kenyan National Malaria Strategy was launched with the management and prevention of malaria in pregnancy as a major component with a stated aim to ensure that 60% of pregnant women were using an ITN or effective IPT by 2006 (MOH, 2001). The Division of Reproductive Health (DRH) of the Kenyan Ministry of Health developed an implementation strategy to ensure the effective clinical management of anaemia and delivery of IPT among Antenatal Care (ANC) attendees with the support of the John Hopkins Programme for International Education in Reproductive Health (JHPIEGO) and financial support from the Department for International Development (DFID), United Kingdom. Pregnant women in

Kenya are given IPT of at least two doses of SP at the antenatal care (ANC) clinics (Gikandi, Noor, Gitonga, Ajanga and Snow, 2008).

In Tanzania, malaria is a major health problem in pregnancy. Malaria is responsible for more than one-third of deaths of children under the age of five years and for up to one-fifth of deaths among pregnant women (Mboera, Makundi and Kitua, 2007). Tanzania adopted the policy of SP for IPT in pregnancy since August 2001 (Tarimo, 2007). The first dose is given in the 20th week of pregnancy and second dose in between the 30th and 36th week under a directly observed therapy so as to improve coverage. Despite the very high rate (80%) of attendance to the Reproductive and Child Health (RCH) clinics in Tanzania, only 29% of mothers would receive and take SP for IPT the picture being similar in other holoendemic settings. A number of socio-cultural factors such as awareness of the benefits, availability and accessibility at the RCH clinics, perceived efficacy and safety; and convenience for use might influence receipt and intake of SP for IPT (Tarimo, Minjas and Bygbjerg, 2001).

In 2005, the Ministry of Health of Burkina Faso adopted IPT with SP to replace weekly chloroquine chemoprophylaxis, whose efficacy had declined because of widespread resistance (Sirima et al., 2003) in pregnancy. IPT with SP is only administered to pregnant women who only present themselves at the ANC clinics.

Nigeria adopted the IPT strategy in 2005 (FMOH, 2005; Efunshile et al., 2011). The National Malaria Treatment Guideline and Policy in Nigeria recommends SP as first line agent for IPT in pregnancy and quinine for treatment of clinical malaria in all trimesters (Akinleye, Falade and Ajayi,

2009). IPT with SP is administered by a health worker at least twice to all pregnant women after quickening and at intervals of 4 weeks, under direct observation (FMOH, 2005).

In Malawi, anti-malarial chemoprophylaxis during pregnancy has been the primary strategy for malaria control in pregnancy for many years with chloroquine initially introduced in 1987. In 1993 sulfadoxine-pyrimethamine (SP) replaced chloroquine with a change from weekly chloroquine prophylaxis to IPT (FMOH, 2005).

IPT-SP policy was adopted in Benin in 2004, but its implementation in the field did not truly begin until 2006 (d'Almeida et al., 2011). A study conducted in Benin from 2004 to 2005 (Briand et al., 2008) proved the efficacy of IPT-SP against IPT-chloroquine (d'Almeida et al., 2011).

IPT with SP in Ghana

Malaria in pregnancy and related morbidity are frequent occurrence in Ghana(Mockenhaupt et al., 2000). Ghana started the implementation of IPT in pregnancy with three recommended doses of SP at the end of 2004 (Hommerich et al., 2007). IPT of malaria during pregnancy is based on the assumption that every pregnant woman living in areas of high malaria transmission has malaria parasites in her blood or placenta, whether or not she has symptoms of malaria. All asymptomatic pregnant women receive regular doses of sulfadoxine-pyrimethamine (SP) as an IPT during the second and third trimesters to clear a presumed burden of parasites, while mothers with signs and symptoms of malaria get prompt treatment according to the national

treatment guidelines. The drug of choice for IPT in Ghana is sulfadoxine-pyrimethamine (SP).

The free antenatal care in Ghana (Arthur, 2012) requires that pregnant women are given malaria prophylaxis in the form of SP tablets obtained from a particular pharmaceutical firm. IPT-SP is given at the antenatal clinic (ANC) or at the where there is supervision of a midwife or health worker through a directly observed treatment (DOT) method. Up to a maximum of three doses is recommended by the Ministry of Health. First dose is given after quickening or 16 weeks, second dose is given at least one month after the first dose and the third dose, given at least one month after the second dose.

Malaria induced anaemia in pregnancy

Anaemia is an important public health problem worldwide and the most vulnerable group, are pregnant women and children (Glover-Amengor, Owusu and Akanmori, 2006). Malaria during pregnancy poses great risks to both mother and child. The most common risk is anaemia. Anaemia increases the mother's risk for placental abruption, pre-term labour and maternal death and puts the developing baby at risk of low birth weight and miscarriage (Czechowicz, Maldonado and Benavente, 2006). Malaria infection in pregnancy is associated with maternal anaemia which, together with placental malaria lead to an increased risk of intra-uterine growth retardation, abortion, pre-term delivery, low birth weight and still births (Steketee, Wirima and Hightower, 1996).

Anaemia in pregnancy is more common in developing countries than in developed countries and is multifactorial in etiology (VanderJagt et al.,

2007). In sub-Saharan Africa, iron and folate deficiencies are the most common causes of anaemia in pregnant women (Baker and DeMaeyer, 1979). The former is related to nutritional deficiency and intestinal helminthic infections and the latter to poor intake of folic acid or food containing folate and chronic hemolytic states. Hemolytic anaemia, is commonly seen during pregnancy in malarious areas of developing countries (Brabin, Hakimi and Pelletier, 2001). The observation that severe anaemia is greatly reduced in patients who have received regular malaria prophylaxis during pregnancy (Fleming, Ghatoura, Harrison, Briggs and Dunn, 1986; Garner and Brabin, 1994; Shulman et al., 1999) indicates that anaemia is related to chronic infection with *Plasmodium falciparum* malaria. In areas of high malaria transmission, *P. falciparum* malaria and anaemia are more frequent in primigravidae (Brabin, 1983). The risk of anaemia is high in teenage primigravidae in developing countries (Arkutu, 1979; Barr, et al, 1998) and developed countries (Beard, 1994; Osbourne, Howatt and Jordan, 1981).

Harrison (1989) has championed the arguments for developing improved pregnancy care to reduce maternal mortality in developing countries. The importance of maternal anaemia as a contributory factor to maternal death has been highlighted (Munasinghe and van den Broek, 2006). In developing countries, where malaria is most common, anaemia is a common feature of pregnancy. Anaemia as presenting feature in pregnancy is more common in partially immune multigravidae living in hyperendemic areas (Azuonwu, Amadi, Wokem and Kalu, 2011). Anaemia in malaria patients could be due to haemolysis of parasitized red blood cells, accelerated removal of both parasitized and unparasitized red blood cells as well as ineffective

22

erythropoiesis with dyserythropoietic changes (Akhtar, Gumashta, Mahore and Maimoon, 2012). Profound haemolysis can aggravate folate deficiency.

Severe malaria with an increase in density of parasitaemia causes anaemia (Anchang-Kimbi, Achidi, Nkegoun, Sverremark-Ekstrom and Troye-Blomberg, 2009).

Anaemia increases perinatal mortality and maternal morbidity and mortality (Conde-Agudelo, Belizan and Lammers, 2005). It also increases the risk of pulmonary oedema (Joubert and Dyer, 2005). Risk of post-partum haemorrhage is also higher (Ferrer, Roberts, Syndendam, Blackhall and Shakur, 2009). Since anaemia is one of the most important consequences of malaria infection during pregnancy, as part of routine antenatal care, every woman should receive iron/folate supplementation (Kawai, Spiegelman, Shankar and Fawzi, 2011). According to World Health Organization all pregnant women should also be screened for anaemia, and those with moderate to severe anaemia should be managed according to national guidelines (WHO, 2004). In malaria-endemic areas, pregnant women with severe anaemia must be treated presumptively with an effective antimalarial, whether or not peripheral parasitaemia and is present or whether or not she has a history of fever (WHO, 2004).

Significant anaemia (haemoglobin less than 7-8g/dl) may have to be treated with blood transfusion. In view of the increased fluid volume in pregnancy, it is better to transfuse packed red cells than whole blood (Salhan, Tripathi, Singh and Gaikwad, 2012).

Low birth weight is still the greatest risk factor for neonatal mortality and a major contributor to infant mortality (Conde-Agudelo, Belizan and

Lammers, 2011). Maternal anaemia is commonly considered a risk factor low birthweight (Ahmad, Kalsoom, Sughra, Hadi and Imran, 2011).

Malaria and parity

The presentation of malaria during pregnancy varies according to the level of transmission in the area (Adam, Khamis and Elbashir, 2005).In areas of high malaria transmission, it has been repeatedly observed that the risk of pregnancy associated malaria decreases in multigravida (Marinho et al., 2009). Marinho et al. (2009) confirmed that the incidence of pregnancy-induced recrudescence, the intensity of the parasitaemia peak and the impact on the pregnancy outcome decreased gradually from the first to the third pregnancy. Malaria infection (peripheral or placental) is more frequent in primigravidae and secundigravidae than in multigravidae and the difference between infected and non-infected women in mean haemoglobin and mean birth weight are more marked in primigrvaidae than in multigravidae (Coulibaly, Gies and D'Alessandro, 2007). The severe clinical manifestations are mainly restricted to the first and second pregnancies (Mendez, 2006) when the levels of specific antibodies against parasites antigens are not high enough to confer protection (O'Neil-Dunne, Archur, Agbor-Enoch, Valiyaveettil, 2001). Interestingly, in areas of infrequent malaria exposure, where women have little or no malaria immunity, the disease results in severe outcomes both for the mother and the baby, irrespective of parity, reinforcing the notion that the severity of clinical manifestations during pregnancy depends on pre-existing immunity of the mother (Mendez, 1995). Thus malaria exposure through consecutive

pregnancies is required for placental malaria immunity to develop and be maintained (Hviid, 2004).

The primigrvaidae has never before been exposed to the placental phenotype of parasites and will allow this infection to proceed. In contrast, as the number of pregnancies increase, the multigravidae will be less and less susceptible to placental infection, possibly by immunity developed due to repeated exposure to the placental phenotype. Each new pregnancy is a booster. This fits well with the progressive decline of malaria in successive pregnancies (Stanton, 1995). Nnaji, Okafor and Ikechebelu (2006) reported that there was a significant difference between the rate of malaria parasitaemia in the primigrvaidae and multigravidae. In the same study, the occurrence of malaria parasites in the placenta was found to decrease with increasing parity. The primigrvaidae had a higher mean parasite density when compared with the multigravidae. It was also demonstrated, that a higher prevalence of malaria with increased parasitaemia occur in pregnant women of lower parity. Incidence of malaria infection in pregnancy is known to be higher in primigravidae than in multigravidae (Graves and Gelband, 2000). Malaria during the first pregnancy causes a high rate of foetal and neonatal death. The decreasing susceptibility during subsequent pregnancies correlates with acquisition of antibodies that block binding of infected red cells to chondroitin sulphate-A (CSA) (Buffet et al., 1999).

Seasonal variation of malaria transmission

Malaria transmission is affected by climate and geography (WHO, 1998). Mandal, Sarkar and Sinha(2011) observed that malaria transmission is

influenced by inter-related factors such as temperature, humidity, rainfall pattern and behaviour of human population. The incidence of malaria and fever are higher in the wet than in the dry seasons. The wetter months may have enhanced mosquito vector breeding and hence an increased malaria transmission (Ojo and Mafiana, 2005). Smedman, Gunnlaugsson, Norby, Silva and Zetterstrom, (1988) reported that malaria transmission is lower in the dry season and peaks in the rainy season, when the parasitaemic rate is greatest. Syafruddin et al., 2009 showed the prevalence of malaria in the wet season and dry season across West Sumba district in Indonesia. The prevalence of *P. falciparum* infection was higher in the wet season than in the dry season. Presumably, the higher prevalence of *P.falciparum* in the wet season is due to increased abundance of the vector mosquito species in this season. According to a research conducted in West Cameroon, malaria parasite was prevalent throughout the year but was significantly higher in the rainy season than in the dry season. Mean parasite density was also higher in the rainy season than in the dry season (Nkuo-Akenji et al., 2006). This is because a high Anopheles species population corresponds with higher malaria parasite prevalence and a higher parasite density (Nkuo-Akenji et al., 2006). In the dry season, the formation of water pools around some public water taps due to poor drainage, coupled with much sunlight was conducive to breeding of Anopheles gambiae (Fontenille et al., 1997; Tadei et al., 1998). High rainfall in the rainy season produces more pools and swamps due to poor drainage, producing suitable conditions for mosquitoes. Thus the persistence of some swamps, together with the existence of bushes that surround many households which serve as resting sites for these mosquitoes during even the dry season leads to high

exposure to mosquito bites and risk of malaria parasite infection all year round (Nkuo-Akenji et al., 2006). The seasonal variation in the biting density of mosquitoes is highest in the rainy months and least in the dry season (Ojo and Mafiana, 2005).

ABO blood groups and malaria

The ABO blood group system is arguably the best known, and yet the most functionally mysterious, genetic polymorphism in humans (Cserti and Dzik, 2007). In clinical practice, ABO is the most important system for blood group compatibility. In the century since their discovery, ABO antigens have been linked with infections and other diseases (Moulds and Moulds, 2000). Some reports found unexpected associations, such as the susceptibility of group A individuals to salivary or gastric cancers (Aird, Bentall and Fraser, 1953). However, the selection pressures defining the ABO distributions remain uncertain. DNA sequence information dates the emergence and development of the group O allele to a period of evolution before human migration out of Africa, concomitant with *P. falciparum*'s activity (Cserti and Dzik, 2007).

P. falciparum has been called the "the strongest known force for evolutionary selection in the recent history of the human genome" (Kwiatkowski, 2005). The signature of *P. falciparum* has taken its toll on human life, especially children. Infectious diseases that kill children select for survival genes and effectively prevent transmission of genotypes unfavourable for survival. In the case of *P. falciparum*, untreated children have a higher case fatality rate than adults. The ability of *P. falciparum* to kill before

reproduction has given it the capacity to select emerging polymorphisms as rapidly as can be witnessed in evolutionary time (Cserti and Dzik, 2007).

Distribution of blood groups

During infection with *P. falciparum*, group O offers a survival advantage, group A confers a disadvantage, and group B has an intermediate effect. Given this hypothesis, one would expect to find that the ratio of group O to A is higher in geographic regions where malaria is currently, or was previously, endemic (Uneke, Ogbu and Nwojiji, 2006). An especially high prevalence of group O coupled with a low prevalence of group A is found throughout sub-Saharan Africa, where *P. falciparum* persists to this day. In the Western hemisphere, the distribution of group A and group O generally matches malaria's tropical distribution. From the tropical regions of Central and South America southward, the indigenous peoples are almost exclusively group O (Cserti and Dzik, 2007). In Asia, the prevalence of group O rises among peoples who live closer to the equator. For example, in Beijing, China (a cold weather zone), group O is 29% and group A 27%, but in Canton, China (a more tropical zone), group O is 46% and group A is 23%. Group O is the most common blood group in Turkey and Persia (O'Neil, 2012). People of African origin possess the O gene more frequently than other races. This lends itself to the possibility that the blood group O gene originated with them.

In contrast, group A is the predominant blood in the colder regions of the earth, where malaria has not been endemic. In fact, group A is found in highest frequency in Scandinavia, Greenland, and the subarctic regions of Europe and North America. There is a higher prevalence of group O observed

in malaria-endemic sub-Saharan Africa compared to many parts of the world where malaria is not endemic, suggesting that blood group O may be a selected, protective adaptation against severe and fatal infection (Martin et al., 1979; Saitou and Yamamoto, 1997).

Blood group O protects against malaria

A number of studies have shown susceptibility to several infectious diseases to be related to the patient's blood group. Malaria has been a major selective force on the human population and several erythrocyte polymorphisms have evolved that confer resistance to severe malaria. Studies of the pathogenesis of malaria have shown that parasite-triggered red blood cell rosette formation is associated with the severity of clinical disease and with cerebral malaria (Pathirana et al., 2005; Ringwald et al., 1993; Uneke, 2007). Rosetting is a known parasite virulence factor that is thought to contribute to the pathogenesis of severe malaria obstructing microvascular blood flow. The rosettes formed by the red blood cells clog up minute blood vessels delivering oxygen to the brain and cause death. Proteins secreted by the parasite turn the red blood cells sticky, forming the "rosettes"Some strains of *Plasmodium falciparum* preferentially trigger rosette formation depending on the red blood cell blood group, with A and B group cells being more likely to form rosettes (Carlson and Wahlgren, 1992; Udomsangpetch, Todd, Carlson and Greenwood, 1993). Studies have shown that rosetting is reduced in blood group O erythrocytes compared with the non-O blood groups (Nasr, Eltoum, Yassin and ElGhazali, 2012; Rowe et al., 2007).

Laboratory diagnosis of malaria

In the laboratory, malaria is diagnosed using the following methods:

Microscopic diagnosis using stained thin and thick peripheral blood smears (PBS)

The accepted laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa, Wright's or Field's stain (Warhurst and Williams, 1996). Although they require a minimal amount of reagents and equipments, these methods of detection and identification depend for their accuracy on well-trained and experienced technologists. Even in the hands of well-trained technologists, diagnosis may be hampered by the sparseness of organisms on the slide and by the subjective nature of differentiating similar-appearing organisms (Rosenblatt, 2009). Blood stained by pricking a finger or earlobe is the ideal sample because the density of developed trophozoites or schizonts is greater in blood from this capillary-rich area (Gilles, 1993). Blood obtained by venipuncture collected in heparin or Sequestrine [ethylenediaminetetraactic acid (EDTA)] anticoagulant-coated tubes is acceptable if used shortly after being drawn to prevent alteration in the morphology of white blood cells and malaria parasites (Moody, 2002). The standard method for diagnosis of malaria is microscopy of stained thick and thin blood films (Rosenblatt, 2009).

The thick blood film concentrates the layer of red blood cells (RBC) on a small surface by a factor of 20 to 30 and is stained as an unfixed preparation using Field's stain, or diluted Wright's or Giemsa stain. The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for detection of low levels of parasitaemia and reappearance

of circulating parasites during infection, recrudescence or relapse. The lysis of the RBC during the staining process can make the process of scanning for parasites more difficult until experience is gained in finding the parasites among the white blood cells (WBC) and platelets (Moody, 2002). The expected sensitivity that can be achieved by an experienced microscopist for the examination of the thick blood film procedure is about 50 parasites/μl of blood (assuming a total RBC count of 5×10^6/μl of blood), which is equivalent to 0.001% of RBC infected (Moody, 2002). Milne, Kyi, Chiodini, and Warhurst, 1994, found that most routine diagnostic laboratories generally achieved a lower sensitivity of detection (average, 0.01% RBC infected, 500 parasites/μl) in an examination of results from British laboratories submitted to the Malaria Reference Laboratory.

Warhurst and Williams (1996) reported that examination of thin blood films is only 1/10 as sensitive as examination of thick blood films for the quantification of malarial parasites, although morphological identification of the *Plasmodium* species present is much easier using thin films. Therefore, most laboratories involved in the quantification and identification of malarial parasites by microscopy produce both thick and thin blood films. It is highly recommended that both thick and thin films be prepared and examined each time blood film examination for parasites is requested.

The thin blood film is methanol fixed and stained with diluted Giemsa or Wright's stain using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC. Because of the fixed monolayer of RBC available in this procedure, the morphological identification of the parasite to the species level is much easier and provides greater specificity than the thick-film

examination. The thin blood film is often preferred for routine estimation of the parasitaemia because the organisms are easier to see and count. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for *P. falciparum* infections (Moody, 2002).

The wide acceptance of this technique by laboratories all around the world can be attributed to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density, are all parameters useful for the management of malaria. The staining and interpretation processes are labour intensive, time consuming and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitaemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels. Although the expert microscopist can detect up to 5 parasites/µl, the average microscopist detects only 50-100 parasites/µl (Payne, 1988).

Quantitative buffy coat (QBC)

The quantitative buffy coat (QBC) technique was designed to enhance microscopic detection of parasites and simplify malaria diagnosis (Clendennen, Long and Baird, 1995). This method involves staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by fluorescent microscopy. Finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube centrifuged at 12 000g for 5 minutes and immediately examined using an epi-fluorescent microscope (Chotivanich,

Silamut and Day, 2006). The principle of QBC technique is based on the fact that on centrifugation at a high speed, the whole blood separates into plasma, buffy coat and packed red cell layer. The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer. Blood cells in the buffy coat layer separate according to their densities, forming visibly discrete bands; platelets remaining at the top, lymphocytes and monocytes within the middle layer and granulocytes at the bottom (Salmani, Mindolli and Peerapur, 2011). Due to acridine orange (Salmani*et al.,* 2011) the malaria parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange (Tangpukdee, Duangdee, Wilairatana and Krudsood, 2009). The tube is examined in the region between the red blood cells and granulocytes and within the granulocytes and mononuclear cell layer, where parasites are most abundant (Salmani*et al.,* 2011). The QBC technique has been shown to be a rapid and sensitive test for diagnosing malaria in numerous laboratory settings (Adeoye and Nga, 2007).While it enhances sensitivity for *P. falciparum*, it reduces sensitivity for non-falciparum species and decreases specificity due to staining of leukocyte DNA (Moody, 2002). Although the QBC technique is simple, reliable and user-friendly, it requires specialized instrumentation, more costly than conventional light microscopy and it is poor at determining species and numbers of parasites (Tangpukdee et al., 2009).

Rapid diagnostic tests (RDTs)

RDTs for malaria are fast and easy ways for determining the presence of malaria parasites. RDTs do not require electricity or specific equipment (Bell et al., 2006). RDTs detect malaria antigen in blood flowing along a

membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or Plasmodium lactate dehydrogenase (pLDH) or aldolase (Bell, Wongsrichanalai and Barnwell, 2006; Murray et al., 2008). Most of the available tests usually involve blotting a small volume of blood (2-20 µl) on a nitrocellulose strip containing monoclonal antibodies, which react with parasite specific antigens available in the blood of infected patients to give visible, diagnostic and control bands (Moody, 2002). Although most RDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria (Lee, Jeon, Jeon and Park, 2008). Recently a new RDT method has been developed for detecting *P. knowlesi* (McCutchan, Piper and Makler, 2008). The accuracy (sensitivity and specificity) of RDTs is mostly dependent on the parasite species, transmission intensity, parasite density, amount of circulating antigens, local polymorphisms of target antigen and persistence of antigens after treatment (Bell et al., 2006, Murray et al., 2008). Results of rapid diagnostic tests are rapidly available, less liable to the theoretical risk of being falsely negative due to parasite sequestration, and visible to both prescriber and patient, and they may result in greater respect for the result (Reyburn et al., 2007). RDTs appear a highly valuable, and rapid malaria-diagnostic tool for healthcare workers; however it must currently be used in conjunction with other methods to confirm results, characterize infection and monitor treatment (Tangpukdee et al., 2009).

Serological tests

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasite. Immunofluorscence antibody testing (IFA) has been a reliable serological test for malaria in recent decades (She et al., 2007). The principle of IFA is that, following infection with any *Plasmodium* species, specific antibodies are produced within 2 weeks of initial infection, and persist for 3-6 months after parasite clearance (Tangpukdee et al., 2009). IFA uses specific antigen or crude antigen prepared on a slide, coated and kept at -30ºC until used, and quantifies both IgG and IgM antibodies in patient serum samples. Titers > 1: 20 are usually deemed positive, and < 1: 20 unconfirmed. Titers > 1: 200 can be classified as recent infections (Chotivanich et al., 2006). Until recently, IFA was a validated method for detecting *Plasmodium*-specific antibodies in various blood bank units, which was useful for screening prospective blood donors, so avoiding transfusion-transmitted malaria (Mungai, Tegmeier, Chamberland and Parise, 2001; Reesing, 2005). IFA is simple and sensitive, but time consuming. It cannot be automated, which limits the number of sera that can be studied daily. It also requires fluorescence microscopy and trained technicians; readings can be influenced by the level of training of the technicians, particularly for serum samples with low antibody titers (Tangpukdee et al., 2009).

Polymerase chain reaction (PCR)

PCR assays used in detecting circulating parasites by demonstrating parasite deoxyribonucleic acid (DNA) through amplification of ribosomal

ribonucleic acid (rRNA) genes represent the overall gold standard of malaria diagnostics. Quantitative PCR can be used to determine the concentration of circulating DNA and therefore estimate the density of circulating parasites (Taylor et al., 2010). PCR-based techniques are a recent development in the molecular diagnosis of malaria and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitaemia or mixed infection (Morassin, Fabre, Berry and Magnaval, 2002). The PCR technique continues to be used extensively to confirm malaria infection, follow-up therapeutic response and identify drug resistance (Chotivanich et al., 2006). It was found to be more sensitive than QBC and some RDTS (Makler, Palmer and Ager, 1998). PCR can detect as few as 1-5 parasites/µl of blood compared with 50-100 parasites/µl of blood by microscopy or RDT. Moreover PCR can help detect drug resistant parasites, mixed infections, and may be automated to process large numbers of samples (Hawkes and Kain, 2007). Although PCR appears to have overcome the two major problems of malaria diagnosis-sensitivity and specificity, the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians (Tangpukdee et al., 2009). PCR is therefore, not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely (Mens, van Amerongen, Sawa, Kager and Schallig,2008). Quality control and equipment maintenance are also essential for the PCR technique, so that it may not be suitable for malaria diagnosis in remote rural areas or even in routine clinical diagnostic settings (Hanscheid and Grobusch, 2002).

Conventional microscopic examination of peripheral thick and thin blood smears remains the gold standard for malaria diagnosis. Although the method requires a trained microscopist, sensitivity and specificity vary compared with recent technical advances, it is inexpensive and reliable (Tangpukdee et al., 2009).

Determination of parasite density

A thick film will contain about 20 times more red blood cells (RBC's) than a thin film, which makes it easier to find parasites (Malaria Consortium, 2007).The thick blood film method gives a parasite count per micro-litre (µl) of blood. The number of parasites in a thick blood film is counted in relation to the number of leucocytes or white blood cells (WBCs). A standard WBC count of 8000 per µl of blood is assumed.

According to Rogier, Commenges and Trape, (1996) clinical malaria is often not associated with parasite densities of *P. falciparum* less than 10,000/µl in Africa and Papua New Guinea. However maximum risk of malaria fever was observed among subjects with parasite density greater than 1000 parasites/µl, though the risk was higher among subjects with parasite density greater than 10,000 parasites/µl, (Delley et al., 2000).

Haemoglobin level and malaria

Haemoglobin is the red colouring matter which forms the most abundant protein constituent of blood. A major physiological function of haemoglobin is to play the role of oxygen carrier in the body, by virtue of its ability to react reversibly with oxygen (Neelakantaswamy, Aspar, Rajaratnam

and Das, 1984). The haemoglobin content in blood determines the vitality of the entire human body, and so haemoglobin monitoring is a necessary clinical procedure for reliable assessment of the current condition of the patient and subsequent prognosis for development of critical conditions in anaesthesiology, emergency care and intensive care (Lisenko and Kugeiko, 2012). Determination of blood haemoglobin concentration as well as the haematocrit and erythrocyte counting is of great importance for screening and diagnosis related to anaemia and erythrocytosis (Suzuki, 1998).

Malarial infection results in decreased haemoglobin concentrations and increased serum concentrations of erythropoietin and transferrin receptor (Verhoef et al., 2002). In a study in Zimbabwe, it was realized that individuals with blood group A had lower haemoglobin levels and to be at greater risk of developing severe central nervous system malaria with coma (Fischer and Boone, 1998). Malaria-induced anaemia, characterized by low haemoglobin levels, has been identified as one of the life-threatening complications of childhood malaria (Asobayire, Adou, Davidsson, Cook and Hurrell, 2001) Malaria is associated with a reduction in maternal haemoglobin (Abrams et al., 2005). The malaria parasite *Plasmodium falciparum,* uses host erythrocyte haemoglobin as a major source of nutrient. It has been estimated that between 25% and 75% of the haemoglobin in an infected erythrocyte is degraded. Therefore in an average patient with about 750g of circulating haemoglobin and a heavy malaria infection at 20% parasitaemia , up to 100g of haemoglobin is utilized during a single cycle (Goldberg, Slater, Cerami and Henderson,1990).

Red blood cell polymorphisms and malaria

Half a billion episodes of *Plasmodium falciparum* malaria occurs each year and result in deaths of more than a million children in sub-Saharan Africa alone. Historically, this burden has resulted in the selection of hundreds, if not thousands, of genetic variants that confer some degree of protection against death from the disease (Mackinnon, Mwangi, Snow, Marsh and Williams, 2005). The Gerbich-negative blood group (Ge-), which results from the deletion of exon 3 in the gene encoding glycophorin C (GYPCΔex3), has long been implicated in malaria resistance because it is found at very high frequencies in malaria endemic regions in Papua New Guinea (Booth and McLoughlin, 1972). It has been shown that in vitro, GYPCΔex3 confers protection against a subset of parasites that use an invasion pathway which involves the *P. falciparum* merozoite erythrocyte-binding antigen 140 (EBA-140) (Thompson, Triglia, Reed and Cowman, 2001)and glycophorin C(GYPC) (Maier et al., 2003). Ovalocytosis, a condition that can result from a number of different genetic lesions, has been considered a strong malaria-protective candidate. Studies conducted in Papua New Guinea have now confirmed that the commonest form, Southeast Asian ovalocytosis, which is caused by heterozygosity for a 27-base pair deletion in the gene that encodes the red blood cell (RBC) membrane protein band 3 (SLC4A1Δ27), is strongly protective against severe malaria and that this protection is highly specific to cerebral form of the disease (Genton et al., 1995). Cockburn et al. (2004) showed that polymorphisms that result in low RBC membrane complement regulatory protein, complement receptor 1 (CR 1) expression are extremely common in malaria endemic regions of Papua New Guinea and are associated

with protection from severe forms of malaria. The haemoglobinopathies, disorders of haemoglobin structure and production, fall into two broad categories: those associated with the production of structurally variant forms of haemoglobin (including haemoglobins S, C and E), and those caused by reduced production of normal forms of either the α-globin or β-globin components of haemoglobin – the α-thalassaemias and β-thalassaemias respectively. The population frequencies of these conditions correlate with the historic incidence of malaria (Flint, Harding, Boyce and Clegg, 1998) and several of the conditions have been shown to protect against various manifestations of clinical *P. falciparum* malaria (Roberts, Harris and Williams, 2004).

Maternal education and fertility

Among the various socioeconomic determinants of fertility, education, especially female education, has received considerable attention from scholars and researchers. In India a 10% increase in the female literacy rate seems to be associated with a 0.5% decline in the total fertility rate (Akmam, 2002). Education (in particular that of women) and residence (rural or urban) are two socioeconomic factors that affect fertility (Akmam, 2002).

It is a recognized fact that female education is an important factor in determining parity even after controlling for related variables such as place of residence (rural or urban), income levels of households and educational levels of husband (Akmam, 2002).Education tends to increase the age at first marriage, thereby decreasing the number of years that can be devoted to child bearing. This relationship between women's education and age at marriage has

been found in almost all fertility studies. A study of 26 developing countries sponsored by the United Nations (UN, 1995) finds that age at marriage invariably increases with the level of education in all of the countries examined, despite the fact that "the age of marriage varies widely across countries". Cleland and Jejeebhoy (1996) showed that in almost every country in South Asia, women with education get married two to five years later than uneducated women.

With education, women become much less fatalistic regarding family size. Cochrane (1978), noted in a study of fertility in Nigeria that only 10% of the women with education beyond the primary stage believed child birth to be determined by God, whereas 50% of the totally uneducated women held that belief. In most research studies it has been found that desired family size becomes smaller with the increase in women's educational levels (Cleland and Jejeebhoy, 1996; Jejeebhoy, 1995; UN, 1995). The dominant behavioural pathway linking education to fertility is the use of contraceptives. With few exceptions, contraceptive use rises steeply across schooling categories (Cleland, 2001).

SP pharmacokineticsand pharmacodynamics

SP is an antimalarial agent, of which a tablet contains 500mg N^1 -(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide (sulfadoxine) and 25mg 2,4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (pyrimethamine) (Roche Pharmaceutical, 2004).

After administration of 1 tablet, peak plasma levels for pyrimethamine (approximately 0.2 mg/L) and for sulfadoxine (approximately 60 mg/L) are reached after about 4 hours (Roche Pharmaceuticals, 2004).

The volume of distribution for sulfadoxine and pyrimethamine is 0.14 L/kg and 2.3 L/kg, respectively. Plasma protein binding is about 90% for both sulfadoxine and pyrimethamine. Both sulfadoxine and pyrimethamine cross the placental barrier pass into breast milk (Roche Pharmaceuticals, 2004).

About 5% of sulfadoxine appears in the plasma as acetylated metabolite, about 2 to 3% as the glucuronide (Roche Pharmaceuticals, 2004). Pyrimethamine is transformed to several unidentified metabolites.

A relatively long elimination half-life is characteristic of both components. The mean values are about 100 hours for pyrimethamine and about 200 hours for sulfadoxine. Both pyrimethamine and sulfadoxine are eliminated mainly via the kidneys (Roche Pharmaceuticals, 2004).

After a treatment dose of SP, plasma concentrations of pyrimethamine (half-life, 3 days) and sulfadoxine (half-life, 7 days) decline log-linearly (Watkins, Mberu, Winstanley and Plowe, 1997). The antimalarial effect depends on synergy between the two components, but the effect from one treatment dose can last as long as 60 days with fully sensitive *Plasmodiumfalciparum* (Watkins, Mberu, Winstanley and Plowe, 1999).

Prevention of malaria with SP

Malaria prophylaxis with SP is not routinely recommended and should only be considered for travelers to areas where chloroquine-resistant *P. falciparum* malaria is endemic and sensitive to SP, and when alternative drugs are not available or are contraindicated (Wyler, 1993) and also for pregnant women.

Drug interaction and untoward effects of SP

There have been reports which may indicate an increase in incidence and severity of adverse reactions when chloroquine is used with SP as compared to the use of SP alone. SP is compatible with quinine and with antibiotics (Roche Pharmaceuticals, 2004). However, antifolic drugs such as sulfonamides, trimethoprim, or trimethoprim-sulfamethoxazole combinations should not be used while the patient is receiving SP for antimalarial prophylaxis.

If signs of folic acid deficiency develop, SP should be discontinued .When recovery of depressed platelets or white blood cell counts in patients with drug-induced folic acid deficiency is too slow, folinic acid (leucovorin) may be administered in doses of 5 to 15 mg intramuscularly daily for 3 days or longer (Roche Pharmaceuticals, 2004). Pyrimethamine was not found in female mice or in male and female rats. The carcinogenic potential of pyrimethamine in male mice could not be assessed from the study because of markedly reduced life span.

Pyrimethamine was found to be mutagenic in laboratory animals and also in human bone marrow following 3 or 4 consecutive daily doses totaling

200mg to 300mg (Roche Pharmaceuticals, 2004). Testicular changes have been observed in rats treated with 105 mg/kg/day of SP and with 15 mg/kg day of pyrimethamine alone. Fertility of male rats and the ability of male or female rats to mate were not adversely affected at dosages of up to 210mg/kg/day of SP. The pregnancy rate of female rats was not affected following their treatment with 10.5 mg/kg/day, but was significantly reduced at dosages of 31.5 mg/kg/day or higher, a dosage approximately 30 times the weekly human prophylactic dose or higher (Roche Pharmaceuticals, 2004).

SP treatment failure

Failure is defined as early treatment failure during the first few days after the start of treatment or the first appearance or persistence of parasitaemia during subsequent follow up (Price et al., 2007). Good quality antimalarial drugs are often misused in treating malaria because of under-dosing and poor adherence, which could lead to treatment failures and development of drug resistance (Onwujekwe et al., 2009). Treatment failure could also be as a result of poor absorption or poor metabolism of the active metabolite (Hombhanje et al., 2005).

Increasing chloroquine resistance necessitated changing the first line antimalarial drug to sulfadoxine-pyrimethamine (SP) in many countries in Africa. Emerging resistance to SP is increasing at an alarming rate. Drug sensitivity tests conducted recently by the East Africa Network for Monitoring Antimalarial Treatment (EANMAT) have shown SP treatment failure rates of $\geq 25\%$ at several sites, the threshold level of drug resistance recommended by WHO for changing an antimalarial drug. Nonetheless, options of alternative

drugs are limited because the few available drugs are either not recommended for use as monotherapy, are expensive, have inappropriate dosage schedule for outpatient use, or have an uncertain safety profile in pregnancy (Mutabingwa, Whitty, Muze, Lemnge and Greenwood, 2003).

SP as prophylaxis in pregnancy

Sulfadoxine-pyrimethamine (SP) has been an alternative to chloroquine for treatment and control of uncomplicated malaria in endemic countries. It was an effective, affordable and complying drug. Sulfadoxine-pyrimethamine (SP) was the first line treatment for *Plasmodium falciparum* infections in many countries; however the clinical efficacy of the combination is decreasing (Gatton and Cheng, 2006). Intermittent preventive treatment with sulfadoxine-pyrimethamine has proven efficacious in reducing the burden of pregnancy-associated malaria but increasing levels of parasite resistance mean that the benefits of national SP-IPT programmes may soon be undermined in much of sub-Saharan Africa (Valley, Valley, Changalucha, Greenwood and Chandramohan, 2007).

The future of SP for intermittent preventive treatment of malaria in pregnancy is tenuous, particularly in East and Southern Africa, and there are insufficient, reliable data on the safety and efficacy of alternative antimalarials for the prevention and treatment of malaria in pregnancy (Valley et al., 2007). Alternative drugs must be found, and new drugs and drug combinations are being recommended by many for deployment as first line treatment at the point that SP resistance forces a policy change. There are however few data on the safety and efficacy of these combinations in pregnant women

(Mutabingwa et al., 2009). It has been suggested that SP should be combined with artemisinins or with cheaperand more readily available alternatives, such as chloroquine or amodiaquine (McIntosh and Jones, 2005) to maintain the effectiveness of SP-IPT in pregnancy or that other drug regimens should be used (White, 2005). Several antimalarials, notably chloroquine, proguanil, mefloquine and proguanil-atovaquone, have been evaluated for malaria chemoprophylaxis in pregnancy (Garner and Brabin, 1994; Schlagenhauf, 1999), but few clinical trials have attempted to evaluate alternatives to SP in IPT in pregnancy regimens. Clinical trials in IPT of malaria in pregnancy have been conducted in Benin, Malawi and Tanzania (National Institutes of Health, 2006) and evaluated SP versus mefloquine; SP alone versus SP plus artesunate; and SP alone versus SP plus azithromycin respectively. Clinical trial among 900 pregnant women in Ghana concluded that amodiaquine alone or in combination with SP was effective in treating uncomplicated falciparum malaria (Tagbor et al., 2006). However, concerns about the safety and tolerability of amodiaquine in pregnancy (Nosten, 2006; Thomas, 2004) and widespread resistance, particularly in East Africa (EANMAT, 2003) are likely to hinder development of amodiaquine-containing combinations for IPT of malaria in pregnancy (Valley et al., 2007).

SP thus becomes the safest and most effective antimalarial for prevention of malaria in pregnancy.

Implications of sub-standard antimalarial drugs

There is little existing knowledge about actual quality of drugs provided by different providers in many sub-Saharan Africa countries. Such information is important for improving malaria treatment that will help in the development and implementation of actions designed to improve the quality of treatment.

A major problem with the treatment of malaria is the high level of treatment failures, resulting in the large part, from the high prevalence of counterfeit drugs (Bate, Coticeli, Richard and Attaran, 2008).

Counterfeiting may apply to both branded and generic products and counterfeits may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient ingredients or with fake packaging (Wondemagegnehu, 1999).

In the Quality of Antimalarials in sub-Saharan Africa study, "substandard" medicines are defined as those that do not meet the quality specifications set for them, primarily because they do not contain the correct amount of the active ingredient(s), do not dissolve properly in the body or include unacceptable levels of potentially harmful impurities (Chinnock, 2010). Substandard drugs may be legitimately produced but poorly stored or transported, too old, or improperly produced, and as a result are of low quality (Tren, 2008).

Antimalarials are among the most widely consumed drugs in tropical countries that have been particularly targeted by counterfeiters (Hall et al., 2006) and are also found in substandard form (Chinnock, 2010).

Counterfeit drugs and substandard drugs are prevalent throughout the developing world. For example a study conducted in Madagascar, Senegal and Uganda on artemisinin-based malaria drugs sold in these countries revealed that about 26 percent to 44 percent "failed quality testing" because of impurities or insufficient amounts of active ingredient (USP, 2010). The three-country report also found bad drugs in both the public and private health sectors (USP, 2010).

Not only are substandard drugs ineffective and sometimes hazardous, they also hasten the development of strains of the malaria parasite that are resistant to the active ingredients (Chinnock, 2010).

Improved drug quality for treatment of malaria will require a concerted educational intervention for providers and consumers to enhance procurement of good quality drugs and improved regulation of the drugs (Onwujekwe et al., 2009).

CHAPTER THREE

METHODOLOGY

Study area

The study was carried out in 7 health centres in 6 selected towns in the Central Region of Ghana. The Central Region is located at the southern part of Ghana. It is bordered by the Ashanti and Eastern regions to the north, Western region to the west, Greater Accra region to the east, and to the south by the Atlantic Ocean. The Central Region is situated within latitude 6º 15 N and 5º N, and longitude 2º 15 W and 45º E. The region is partitioned into 13 districts (fig.1). The region has an area of about 9.826 km² (Opoku, 2009) and a population of about 1.6million (Ovenseri-Ogbomo and Omuemu, 2010). Central region is made up of two ecological zones; the coastal and forest zones. Towns from which subjects were selected are Assin Foso, Twifo Praso and Abura Dunkwa located in the forest zone and Cape Coast, Saltpond and Elmina in the coastal zone. The populations of the towns are Cape Coast: 82,292, Saltpond: 16,212, Elmina: 21,103, Assin Foso: 22,837, Twifo Praso: 9,011 and Abura Dunkwa: 8,439. Fishing, farming and petty trading are the main occupation of the inhabitants. Two rainy seasons occur in the Central region. The main wet season is from April-September and the dry season is from November-March (www.travel-to-discover-ghana.com). The study was conducted in the two seasons; March in the dry season and August in the rainy season in the year 2009.
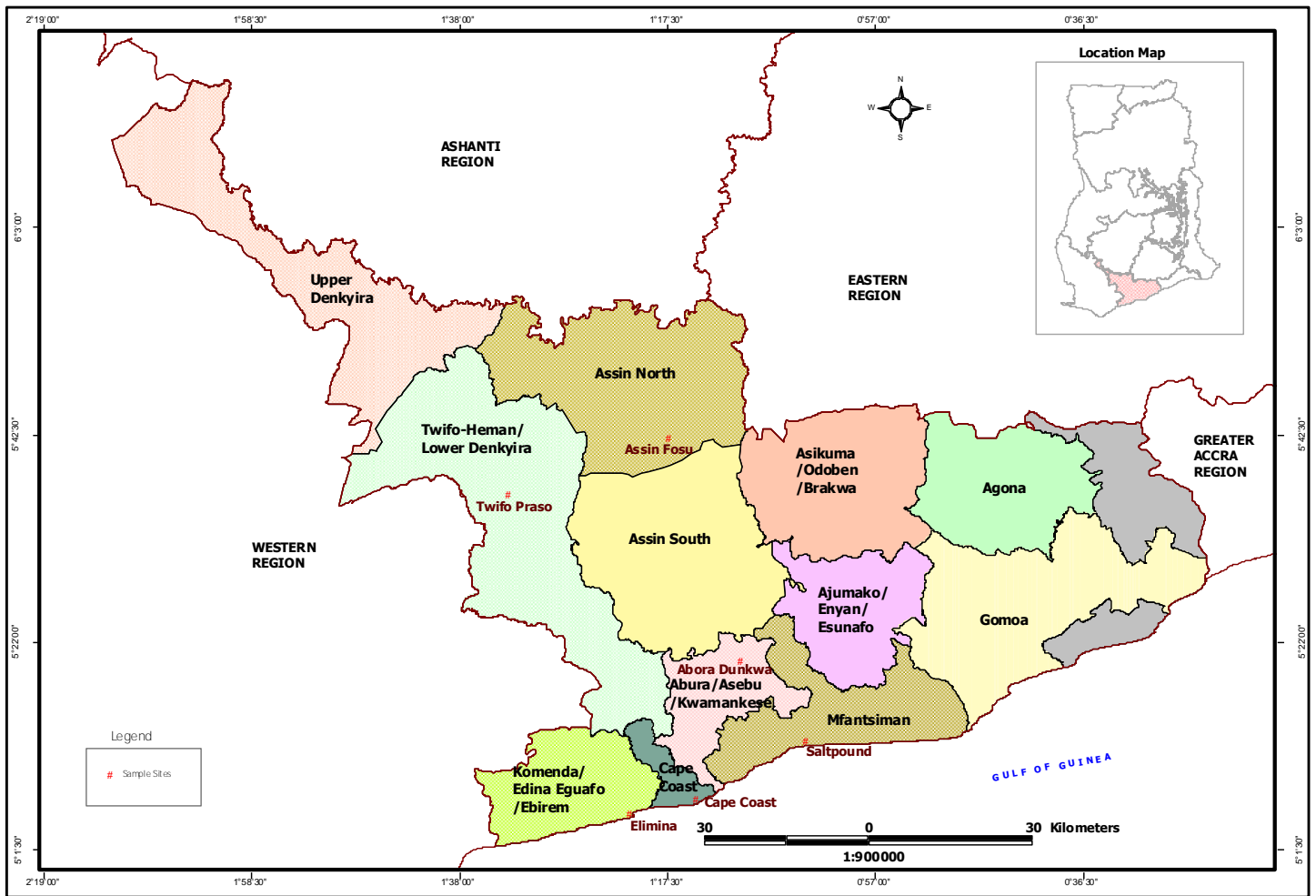
Fig. 1 Map of Central Region of Ghana showing districts

Study population

The study was carried out on pregnant women who had reported at the antenatal clinics of Cape Coast Metropolitan Hospital, Ewim Urban Health Centre at Cape Coast, St. Francis Xavier Hospital at Assin Foso, Twifo Praso Government Hospital, Elmina Urban Health Centre, Saltpond District Hospital and Abura Dunkwa District Hospital for their routine monthly antenatal visits. The hospitals were selected because they were the district hospitals located in the district capitals. A total of 598 pregnant women were enrolled on to the study; 119 from Cape Coast Metropolitan Hospital, 77 from Ewim Urban Health Centre, 85 from St. Francis Xavier Hospital, 88 from Twifo Praso

50

Government Hospital, 84 from Elmina Urban Health Centre, 104 from Saltpond District Hospital and 41 from Abura Dunkwa District Hospital. The sample size was calculated from Fischer and Boone, (1998) (Appendix 3).

Study design

A target population in this cross-sectional study was selected at the antenatal care clinics (ANCs) of the various health centres. The subjects were randomly selected on the basis of being pregnant and present at the ANCs at the time of sample collection. The subjects were recruited in the dry and wet seasons. The demographic characteristics of the pregnant women such as age, educational level, occupation, etc. were obtained through the administration of questionnaire (Appendix 4). Level of maternal education was categorized as follows: Low: no education or primary school, Mid: junior, senior high, vocational and technical schools and High: tertiary education, that is all levels beyond secondary education (van Rossem et al., 2009). Venous blood was taken from the pregnant women by a licensed medical technologist, after endorsing a consent form. The blood was analyzed at the laboratory for malaria parasites, haemoglobin, sickling and blood group. The pregnant women were also classified as primigravidae (woman experiencing first pregnancy) and multigravidae depending on the number of babies delivered. The parasite density was put into four levels; 0, 1-999, 1000-9999 and > 10000 (Obonyo, 2006). Blood groups were determined for A+, A-, B+, B-, AB+, AB-, O+ and O- by standard haemagglutination techniques.

Inclusion criteria

Pregnant women of different parities and gestational period who reported at ANC at any of the selected health facilities and consented to participate were included.

Exclusion criteria

All pregnant women who refused to sign or thumbprint the consent form were excluded. All those who were sickling positive were also excluded.

Ethical consideration

Ethical approval was obtained from the Central Regional Directorate of the Ghana Health Service. The rationale of the study was explained to the subjects with the assistance of the midwives. They were made to understand that they were not obliged to participate in the study and if they opted out, they would still be attended to by the midwives. Some of them opted out primarily because of the pain associated with the drawing of the venous blood. Others also decided not to participate in the study because they were not sure if their blood would not be used for something else, apart from the laboratory analysis. Those who agreed to participate in the study were made to endorse consent forms before they were included in the study.

Sample collection

Venous blood of about 5ml was collected from each of the subjects. The blood was collected by a licensed medical laboratory technologist. The blood was collected by first tying the upper arm. The spot of upper arm where

the blood was drawn was swabbed with 70 percent alcohol using cotton wool and made to dry. The patient was made to clench her fist to make the veins more visible. The median cubital vein was punctured using a needle and about 5ml of blood was drawn. After drawing blood, sterile cotton was put on the wound and the patient's lower arm is raised to stop flow of blood. Blood is poured into EDTA tubes (tubes containing anticoagulant).

Preparation of thick blood film

A thick blood film was made by smearing a drop of blood over a small area of a slide and allowing it to dry. The slide was immersed in a freshly prepared 5% Giemsa stain solution for about 20 to 30 minutes and then flushed with tap water and left for about 30 minutes to dry.

Microscopic examination of slides

The dry slides were observed under the light microscope using x 100 objective lens (Adefioye, Adeyeba, Hassan and Oyeniran, 2007). A drop of immersion oil was first put on the thick film before the observation. The presence of brown-black pigment is a good indicator of parasites (Malaria Consortium, 2007). Parasite density was determined by counting the number of malaria parasites per 200 leucocytes or white blood cells (WBCs). A standard WBC count of 8000 per micro-litre (μl) of blood is assumed.

The number of malaria parasites was counted and the number of WBCs in each field was also recorded. The parasites were counted against 200 leucocytes.

If less than ten parasites are counted after identifying 200 WBCs, counting of parasites continues until 500 WBCs are identified. Parasite number is then recorded per 500 WBCs. A slide was considered negative if no parasite was found after counting 500 WBCs (Coulibaly, Gies and D'Alessandro, 2007). The parasite density or parasite count per µl of blood is calculated using the following formula:

$$\frac{\text{number of parasites counted}}{\text{number of white blood cell}} \times 8000 = \text{parasites per µl}$$

The number of white blood cells is either 200 or 500.

Parasite density was categorized as follows: 0 (aparasitaemic), 1-999, 1000-9999, and ≥10,000 (Obonyo, 2006). Such a classification is appropriate as higher parasite densities correspond to greater severity of the infection and acute phase of malaria (Maombi, Jeeva and Singh, 2011).

Determination of haemoglobin

The haemoglobin content of each blood sample was estimated using Drabkins reagent. About 5ml of Drabkins reagent, which is a solution comprising of Potassium ferricyanide, Potassium cyanide and Sodium bicarbonate, is put into test tube. About 0.2ml of blood was pipetted and put into the test tube containing the Drabkins reagent. The solution is made to stand for about 10 minutes. It is then poured into a cuvette and then inserted into a spectrophotometer. The optical density (absorbance) of the solution was recorded. The iron content of the solution is determined by measuring the absorption of the solution against a standard curve of known iron concentrations. This gives the haemoglobin level of the blood. The

haemoglobin levels were based on the World health Organization standards. Pregnant women with haemoglobin levels below 11g/dl were considered to have iron deficiency anaemia (WHO, 2011). Haemoglobin levels were classified either as normal or low (anaemic).

Determination of sickling

Sickling status of recruited subjects was determined using sodium metabisulphite also referred to as sickling fluid. Using a pipettor, a drop of blood was put on a microscope slide. An equivalent volume of sodium metabisulphite was added. They were then mixed on the slide and a cover slip put on it, and made to stand for about 30 minutes before observation under the microscope (Nsimba et al., 2012). The sodium metabisulphite deoxygenated the red blood cells. The cells containing defective haemoglobin assumed a sickle shape. The observed slides were classified either as sickling positive or negative.

Determination of blood groups

According to the ABO blood typing system there are four different kinds of blood types: A, B, AB or O. A person with blood group A has A antigens on the surface of their RBCs and B antibodies in their blood plasma. Blood group individuals have B antigens on the surface on their RBCs and A antibodies in their blood plasma. Blood group AB individuals have both A and B antigens on the surface of their RBCs and no A or B antibodies in their blood plasma. Blood group O individuals have neither A or B antigens on the surface of their RBCs but have both A and B antibodies in their blood plasma.

The various blood group types are determined by two ways:

1. Forward grouping: The testing of red cell suspensions of unknown group to determine the presence or absence of A and or B antigens. 2. Reverse grouping: Testing the unknown serum or plasma to determine the presence or absence of antibodies corresponding to the antigens lacking on the red cells.

Several methods for testing ABO group of an individual exist but the most common method is Serology. This is a direct detection of the ABO antigens. It is the main method used in blood transfusion centres and hospital blood banks. In blood group serology, the interaction between the antigen sites on the cells and the corresponding antibody is detected by observing agglutination (clumping). Agglutination is the result of the cross- linking of individual red cells by antibody molecules.

ABO antisera used for the forward grouping are anti-A and anti-B reagents. They are both monoclonal antibodies, immunoglobulin M and highly specific. Anti-A is a blue coloured reagent whilst anti-B is a yellow coloured reagent. When a drop of either anti-A or anti-B antisera is put in a red cell suspension, the following reactions are observed:

1. Group A – agglutination with anti-A reagent.

2. Group B – agglutination with anti-B reagent.

3. Group AB - agglutination with anti-A and anti-B reagents.

4. Group O – no agglutination with anti-A and anti-B.

The forward grouping method was used. Three reagents were used: antisera anti-A, anti-B and anti-D. Three drops of blood from a subject were put on a white tile. A drop of reagent A was put on the first blood drop, reagent B on the second blood drop and reagent D on the third blood drop. The blood was mixed with the reagent with applicator stick. The white tile was

then tilted gently and then observed for agglutination. Agglutination with anti-D indicates Rhesus factor (Rh) positive. The blood samples were classified as A-, A+, B-, B+, AB-, AB+, AB-, O- and O+.

Drug analysis

Sulfadoxine-pyrimethamine treatment failure may be caused by parasite resistance or poor quality of the antimalaria drug. In assessing the quality of the SP, the chemical or biological assay and the dissolution methods were employed. Three SP drugs named Drug I (locally made) and given to pregnant women at the antenatal care clinics as prophylaxis, Drug II (locally made) and Drug III (foreign made) commonly found in pharmacies and chemical shops were analyzed at the laboratory of Ghana Standard Board. The batch numbers for the drugs were Drug I: 051-LI0677, Drug II: 007 and Drug III: Z8003. The original names of the drug have been withheld for confidentiality.

Sample preparation for drug assay and drug dissolution

Reference standard solution of SP was prepared by dissolving approximately 0.5g (500mg) of sulfadoxine and 0.025g (25mg) of pyrimethamine in 10ml methanol and diluted with mobile phase to 100ml. System suitability must be demonstrated before starting an analysis to determine the effectiveness of a final operating system. Five replicate injections of a system suitability preparation (standard solution) were chromatographed and the peak areas recorded (Braun, 2005). The calculated relative standard deviation of the peak area of each of the reference i.e pyrimethamine and sulfadoxine was less than 2.5%. According to the USP

2007, the relative standard deviation of each of the reference should not be more than 2.5% to pass system suitability test.

Twenty tablets of each brand of SP drug were weighed and the average weight determined. The 20 tablets of each sample were powdered separately with mortar and pestle. An accurately weighed amount of the finely ground powder, equivalent to 0.5g sulfadoxine and 0.025g pyrimethamine of each sample was dissolved in 10ml of methanol with the aid of an ultrasonic bath. It was then diluted with mobile phase to 100ml, mixed, and filtered (Braun, 2005). The samples were then introduced into the HPLC. Three replicates of each sample were run and the average peak area recorded (Appendix 5). The weight in milligrams of sulfadoxine and pyrimethamine in a tablet of each brand was determined. Using these results, the amounts of sulfadoxine and pyrimethamine per tablet were calculated and expressed as percentage of the label claim (Amin and Kokwaro, 2007).

Tablet dissolution was performed in the ERWEKA DT 600 dissolution apparatus using 6 tablets of each product. Dissolution of the antimalarial products was carried out using 1 litre of 0.01 M pH phosphate buffer solution (sodium hydroxide and potassium dihydrogen orthophosphate, Fisher Scientific) and heated to a temperature of 37º C, with a rotor speed of 75 revolutions per minute (rpm). The 6 tablets were introduced into the dissolution vessels and dissolution was carried for 30 minutes (Onwujekwe et al., 2009). Samples (2ml) of the dissolution media in the different vessels were withdrawn after 30 minutes and was transferred into HPLC reaction vial and then introduced into the HPLC machine for analysis for sulfadoxine and

pyrimethamine content according to the HPLC method described in the USP for dissolution testing of these tablets (WHO, 2003).

Data analysis

Data collected were recorded into a pre-coded case record forms. Thereafter, the data were transferred to Statistical Package for the Social Sciences (SPSS) version 10 for analysis. Descriptive statistics such as means and standard deviations were used to summarize quantitative variables while categorical variables were summarized with proportions. The student t-test was used to compare two mean values while the one way analysis of variance (ANOVA) was used to compare mean values in more than two groups. The Chi-square test was used to investigate associations between categorical values and also to analyze differences in proportions. For significant associations 95% confidence intervals were computed. A p-value of less than 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

Socio-demographic characteristics of the study population

A total of 598 pregnant women were recruited into the programme. Sickling positive pregnant women were 55 (9.2%) and were subsequently excluded from the programme. The ages of the participants ranged from 16 to 44 of which majority 444(82%) of the women were between the ages 18 and 34 years. The pregnancies were from 2-9 months. Majority, 427(79%) of the women had pregnancies between 5-9 months. Only 21% (116) of the women had pregnancies between 1-4 months. The women who were pregnant for the first time constituted 32% (172) whilst 68% (371) of them have had between 1 and 9 pregnancies. About 77% of the subjects were employed.

Malaria parasite infection among pregnant women

Malaria parasites were found in the peripheral blood of pregnant women who had taken SP and those who had not taken. Of the 543 pregnant women, 304 representing 56.0% of them had taken Drug I (SP). Those who had not taken SP were 239 representing 44.0%. A total of 63 pregnant women out of the 543 constituting 11.6% had malaria parasites in their blood. Out of the 63 infected pregnant women 34(54.0%) had taken SP and 29(46.0%) had not taken SP (Table 1). There was no significant difference between those who

had taken SP and yet infected, and those who had not taken SP and infected ($\chi^2 = 0.27$; P value $= 0.871$).

Haemoglobin levels among pregnant women

Maternal anaemia is a risk for both the pregnant woman and the developing foetus. Pregnant women are prone to anaemia. The cause of anaemia in pregnant women is multifactorial. This study was to ascertain the contribution of malaria to the low haeoglobin levels in pregnant women.

The haemoglobin levels of the pregnant women were generally low. As many as 399(73.5%) of the pregnant women had low haemoglobin whilst 144(26.5%) had normal haemoglobin. Forty nine pregnant women representing 77.8% of the infected pregnant women had low haemoglobin (below 11g/dl) whilst 72.9% of the uninfected pregnant women also had low haemoglobin (Table 1). There was no significant difference in the haemoglobin levels of the pregnant women who had malaria parasites in their blood and those who had no malaria parasites in their blood ($\chi^2 = 0.351$; P value $= 0.553$).

Table 1: Educational level, malaria status, haemoglobin level and blood groups among the pregnant women

i. Malaria status among pregnant women who had taken SP or not

MP status	SP Yes	SP No	Total	χ^2	P value
	n(%)	n(%)	N(%)		
Infected	34(54.0)	29(46.0)	63(11.6)		
Not infected	270(56.3)	210(43.7)	480(88.4)		
Total	304(56.0)	239(44.0)	543(100)	0.027	0.871

ii. Haemoglobin level among pregnant women who had malaria infection or not

MP status	Low Hb	Normal Hb	Total	χ^2	P value
	(<11g/dl)	(>11g/dl)			
	n(%)	n(%)	N(%)		
Infected	49(77.8)	14(22.2)	63(11.6)		
Not infected	350(72.9)	130(27.1)	480(88.4)		
Total	399(73.5)	144(26.5)	543(100)	0.351	0.553

iii. Infection status among the blood groups

Blood group	Infected	Uninfected	Total	χ^2	P value
	n(%)	n(%)	N(%)		
O	35(11.6)	265(88.4)	300(55.2)		
Non-O	28(11.5)	215(88.5)	243(44.8)		0.555

iv Educational level between the parities

Educational level	Primigravida	Multigravida	Total	χ^2	P value
	n(%)	n(%)	N(%)		
Low	33(16.5)	167(83.5)	200(36.8)		
Mid	140(42.8)	187(57.2)	327(60.2)		
High	11(68.8)	05(31.2)	16(03.0)		
Total	184(33.9)	359(66.1)	543(100)	0.000	0.000

a)SP (sulfadoxine pyrimethamine); b)MP (malaria parasite);
c)Hb (Haemoglobin level)
Chi-square test
P-value based on chi-square test.

62

Parasite density between primigravida and multigravida

There was relatively greater parasite density in the multigravids than in the primigravids. The primigravidae were 33.9 % (184) and 66.1 % (359) were multigravidae. The infected primigravids and multigravids were 25 (39.7%) and 38 (60.3%) respectively. Forty five infected pregnant women had parasite densities within 1-999 parasites/µl. Out of this number, 16(35.6%) were primigravids compared to 29(64.4%) who were multigravids. Eighteen of the infected pregnant women had parasite densities between 1000-9999 parasites/µl and 9(50.0%) were primigravdae and 9(50.0%) were multigravidae (Table 2). The difference in the parasite densities between primigravidae and multigravidae was not significant ($\chi^2 = 256.0$; *P* value = 1.000).

Table 2: Comparison of malaria parasite density between primigravids and multigravids

	Primigravida	Multigravida	Total	χ^2	*P* value
	n(%)	n(%)	N(%)		
i. MP status					
Not infected	159(33.1)	321(66.9)	480(88.4)		
Infected	25(39.7)	38(60.3)	63(16.6)		
ii. Parasite density					
0	159(33.1)	321(66.9)	480(88.4)		
1 - 999	16(35.6)	29(64.4)	45(08.3)		
1000-9999	9(50.0)	9(50.0)	18(03.3)		
≥ 10000	0(0)	0(0)	0(0)		
Total	184(33.9)	359(66.1)	543(100)	256.0	1.000

MP (malaria parasite)
Chi-square test
P-value based on chi-square test

Seasonal variations in parasite densities among pregnant women

Blood samples were taken from 239(43.9%) pregnant women in the dry season whereas 304(56.1%) pregnant women provided blood samples during the wet season. Malaria parasites were detected in 18 (28.6%) of the pregnant women during the dry season; 17(37.8%) had parasite densities between the range1-999 parasites/µl whilst only 1(05.6%) had parasite densities between 1000-9999 parasites/µl. Infected pregnant women during the wet season were 45 (71.4%) with 28 (62.2%) having parasite densities between 1-999 parasites/µl and 17 (94.4%) having parasite densities between 1000-9999 parasites/µl (Table 3). The parasite densities observed in the blood of the pregnant women were high in the wet season than in the dry season. The difference in the parasite densityamong the infected pregnant women between the two seasons was significant (χ^2 = 53.663, P value = 0.047).

Table 3: Seasonal variations of malaria parasite density in the pregnant women

value	Dry season n(%)	Wet season n(%)	Total N(%)	χ^2	P
i. MP					
Not infected	221(46.0)	259(54.0)	480(88.4)		
Infected	18(28.6)	45(71.4)	63(16.6)		
ii. Parasite density					
0	221(46.0)	259(54.0)	480(88.4)		
1 - 999	17(37.8)	28(62.2)	45(08.3)		
1000-9999	1(05.6)	17(94.4)	18(03.3)		
$\geq$ 10000	0(0)	0(0)	0(0)		
Total	239(43.9)	304(56.1)	543(100)	53.663	0.047

MP (malaria parasite)
Chi-square test
P-value based on chi-square test

Distribution of the blood groups and malaria

Blood group O pregnant women were predominant among the 543 subjects (Fig. 2). Blood group O constituted as many as 55.2% (300) whilst blood group AB was the least making up 4.0% (21). Blood group A formed 22.8% (124) and blood group B, 18.0% (98).
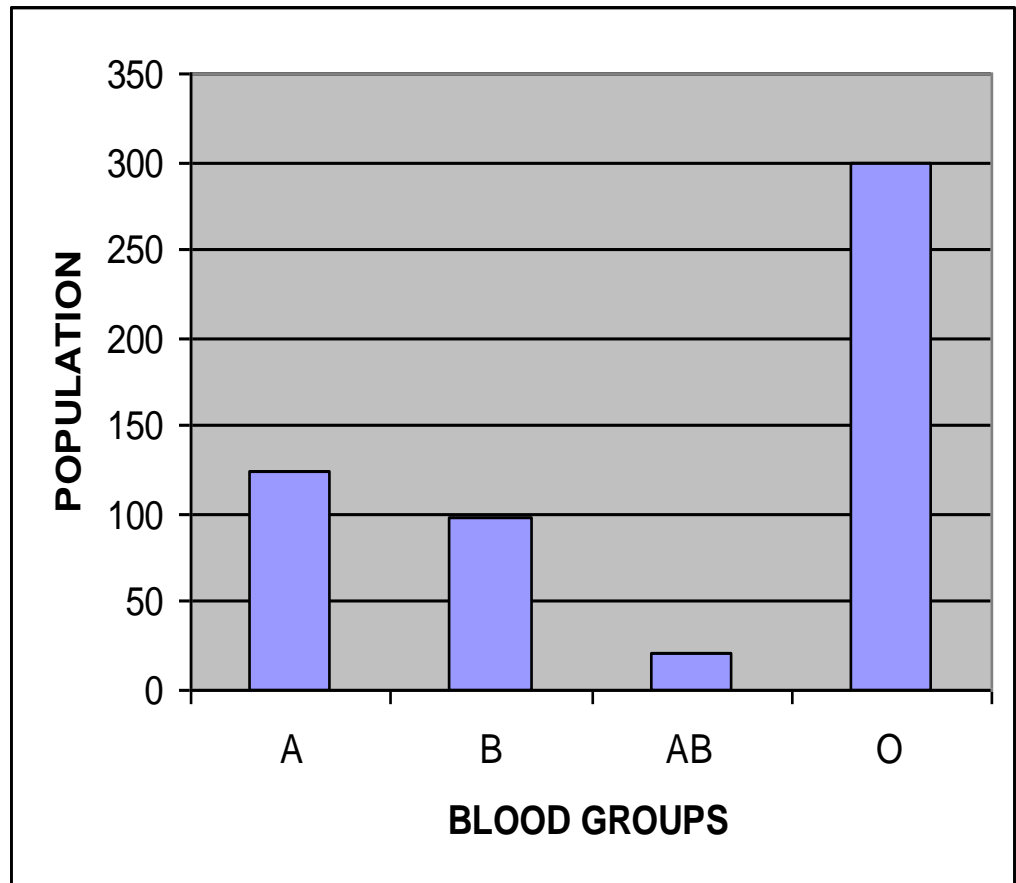
Fig. 2: Frequency of the blood groups of the pregnant women

A total of 243(44.8%) of the pregnant women were non-O blood group individuals whilst 300(55.2%) had blood group O. The non-O blood group individuals who were infected formed 11.5% of the non-O pregnant women whilst 11.6% of the blood group O pregnant women were infected (Table 1). The difference between the infected non-O blood group individuals and the infected blood group O individuals was not significant (P value = 0.555).

Though there were relatively fewer individuals in blood group O with malaria infection than in blood group B and AB, the differences were not significant (Table 4).

Table 4: Comparison of the effect of blood group O with the effect of non-O blood groups on resistance to malaria

Blood groups	P value
O vrs. A	0.086
O vrs. B	0.425
O vrs. AB	0.787
O vrs. non-O	0.555

Maternal educational level and parity

About 68.8% of the pregnant women who had high education were primigravids (Table 1). There was a significant difference in the maternal educational levels of the primigravids and multigravids ($\chi^2 = 0.000$; P value < 0.001).

SP drug test

Drug I and Drug III passed the drug assay test, in that the average percentage composition of both sulfadoxine and pyrimethamine in these drugs fell within the 90.0 % - 110.0% range. Drug II failed the assay test because the average percent composition of pyrimethamine in it, was below 90.0 percent (Table 5). Drug dissolution test was not performed for Drug II, because it failed the first test. Drug III passed the drug dissolution test. The percentage of both sulfadoxine and pyrimethamine of Drug III dissolved in the phosphate buffer in each of the vessels was not less than 60.0 percent. Drug I failed the

drug dissolution test. The percentage of pyrimethamine of Drug I dissolved in the phosphate buffer in each of the vessels was less than 60.0 percent (Table 6). Moreover the percentage of sulfadoxine of Drug I dissolved in the phosphate buffer in two of the vessels was less than 60.0 percent.

Table 5: Calculated weight in milligram and percentage composition of sulfadoxine and pyrimethamine in Drugs I, II and III

Percentage pyrimethamine	Weight of Composition	Percentage sulfadoxine per Composition	Weight of Composition	Percentage pyrimethamine Composition
Drug I	tablet (mg)	(%)	per tablet (mg)	(%)
1	485.72	97.14	21.88	87.52
2	490.36	98.07	23.92	95.68
3	489.52	97.90	23.78	95.12
Average		97.71		92.76
Drug II				
1	485.31	97.06	18.39	73.56
2	485.88	97.18	18.89	75.56
3	485.62	97.90	19.16	76.64
Average		97.12		75.24
Drug III				
1	497.76	99.55	22.51	90.04
2	498.00	99.60	23.72	94.80
3	498.76	99.75	22.85	91.40
Average		99.63		92.12

mg (milligram): % (percentage)
SP tablets should contain not less than 90.0% and not more than 110.0% of sulfadoxine and not less than 90.0% and not more than 110.0% of pyrimethamine.

Table 6: Recorded peak responses of five replicate injections and calculated percentage dissolution of sulfadoxine and pyrimethamine in Drugs I and III

	Average peak area of sulfadoxine (mV)	Percentage of sulfadoxine dissolved (%)	Average peak area of pyrimethamine (mV)	Percentage of pyrimethamine dissolved (%)
Drug I				
1	6892774	59.42	90182	20.20
2	7076779	61.00	114852	25.74
3	6991092	60.27	90667	20.31
4	7017232	60.49	105682	23.67
5	6981546	60.18	110563	24.76
6	6889575	59.39	115472	25.86
Drug III				
1	10898130	93.94	358382.5	80.27
2	10516241	90.65	347357	77.80
3	10790608	93.02	305035	68.32
4	10811924	93.20	314093.5	70.35
5	10679338	92.06	298327.6	66.82
6	10592611	91.31	306223.3	68.59

mV (millivolts)

Not less than 60% of the amount of each of sulfadoxine and pyrimthamine is dissolved in 30 minutes.

CHAPTER FIVE

DISCUSSION

SP treatment failure

It was observed that a greater percentage of the subjects had taken SP. However, there was no significant difference between those who had taken SP but were infected and those who had not taken SP and were infected. This development suggests that Drug I may not be effective. The ineffectiveness of a drug could be due to either development of parasite resistance to the drug or inadequate drug levels, through sub-optimal dosing, poor quality of the antimalarial, poor absorption or poor metabolism to the active metabolites (Barnes, Sibley and Plowe, 2007). Drug assay and the dissolution test conducted on the SP, revealed that the drug could not pass the dissolution test. This explains why the SP could not prevent malaria infection among the pregnant women.

Maternal malaria and anaemia

Generally, the pregnant women were anaemic. Anaemia is one of the most important consequences of malaria infection during pregnancy. Anaemia during pregnancy can cause low birth weight and low birth haemoglobin in the infant. This consequently may lead to increased morbidity for the infant (Chimsuku et al., 1994). Relatively, there was no significant difference between the haemoglobin levels of pregnant women who had malaria infection

and those who did not. This shows that irrespective of malaria, the haemoglobin levels of the pregnant women were generally low. This confirms the fact that malaria is not the sole determinant of anaemia in pregnancy but its etiology is multifactorial, which could also be due to iron deficiency, folate deficiency, poor diet, hookworm infections etc.(WHO, 1986). Two other known factors which contribute to development of iron deficiency anaemia in pregnancy is first the woman's iron stores at the time of conception and the second is the amount of iron absorbed during gestation (McMahon, 2010). There is a normal reduction in haemoglobin level at the beginning of pregnancy followed by a slight rise towards the end of pregnancy (Reveiz, Gyte and Cuervo, 2007). The initial reduction has been explained to result from increased red cell mass and demands of the foetus which exceeds iron intake with consequent reduction in iron stores of the woman's body (Reveiz et al., 2007). This is why iron supplementation in pregnancy has become a standard and routine practice as a preventive treatment for iron deficiency anaemia in pregnancy in developing countries (Osungbade and Oladunjoye, 2012).

Parity and malaria

Most studies suggest that malaria infection is more prevalent in primigravid women than in multigravid in areas endemic for malaria such as sub-Saharan Africa (Brabin, 1983; McGregor, 1984). However in this study it was observed that peripheral malarial infection was comparatively greater in the multigravids than in the primigravids. Parasite density was also higher in

the multigravidae than in the primigravidae. However, the difference between the parasite densities of the two groups was not significant.

Evidence of malaria infection in pregnancy is not only obtained from parasite count in the peripheral blood during pregnancy but also placental infection at the time of delivery (Brabin, 1983). Parasite densities in placental infections are sometimes difficult to assess with accuracy because there seem to be no correlation between parasite density in peripheral blood and in the placenta in pregnant women in malaria-holoendemic regions (Brabin, 1983). Thus, the placenta may contain large numbers of infected red blood cells (as many as 65%), whereas the peripheral blood is free from parasites (Brabin, 1983). Primigravids lack the specific immunity to placental malaria which is acquired from exposure to malaria parasites during pregnancy (Staalsoe et al., 2004). It is therefore possible that in this study there were more parasites in the placenta of the primigravids than in their peripheral blood which goes to confirm the general notion that primigravids are more susceptible to malaria infection.

Seasonal variations in malaria parasite densities

There weremore malaria infections in the rainy season than in the dry season. The parasite densities were found to be significantly higher in the rainy season as compared to those found in the dry season. Rainy seasons are periods when the breeding places of the *Anopheles* mosquito abound. The population of mosquitoes in the environment would increase and this may lead to increase in the frequency of mosquito bites. This may also lead to increase in malaria parasite densities. This finding therefore supports the claim by

Bouvier et al., (1997), that levels of parasite density are higher in the rainy season compared with the dry season.

A,B,O blood groups and malaria

There were more individuals with blood group O than with the non-O blood groups in accordance with observations of Cserti and Dzik (2007). Blood group O had an influence on the malaria status of the pregnant women but it was not significant. Comparing blood group O to B and AB, there were relatively fewer pregnant women with malaria infection among group O than among group B and AB. However, group A had the least pregnant women with malaria infection. Blood group O may therefore provide some protection from malaria infection as have been purported Rowe et al., (2007). There is need for extensive investigations to be carried out in several epidemiological settings with different levels of malaria endemicity to better evaluate the effects of the ABO blood type on malaria.

Maternal education and parity

The study revealed that maternal educational level had a significant influence on the fertility rate of the pregnant women. Women with high education had lesser number of children compared to those with low education. This confirms findings by Cleland and Jejeebhoy (1996) that family size becomes smaller with the increase in women's educational levels. Most women with higher education may not marry early which will lead to delay in the years of giving birththereby reducing their reproductive years. They also

have knowledge of the advantages of spacing children and planning family size. Unwanted pregnancies are prevented by the use of contraceptives.

SP drug test

In Ghana, the government provides free antenatal care. Pregnant women are given malaria prophylaxis in the form of SP tablets (Drug I) obtained from a particular pharmaceutical firm. SP from other sources are sold in Pharmaceutical shops. Drug I and Drug III passed the drug assay test, implying they contain the right quantities of the active ingredients; sulfadoxine and pyrimethamine. However, only Drug III passed the drug dissolution test. The test for dissolution determines the amount of active ingredient that is released and available for absorption. Poor manufacturing practices, poor storage of a product as well as the use of incorrect excipients will lead to poor dissolution profiles (Onwujekwe et al., 2009). The failure of Drug I to pass the drug dissolution test is an indication that a lesser amount of the drug is able to dissolve within the specified period, and thus results in poor absorption, rendering the drug ineffective. This situation exposes the pregnant women to the risk of malaria infection thereby defeating the aim of IPT in Ghana. This may account for the observed persistent malaria infection in pregnant women who have taken SP (Drug I), confirming the hypothesis that SP is incapable of preventing malaria in the pregnant women.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Drug I could not bring about a substantial decline in malaria infection among the pregnant women after the administration of the SP. This might be because the drug was found to be substandard. It failed to conform to quality standards by failing dissolution test. Some of the pregnant women did not receive IPT-SP because of either unavailability of the drug or they were not due for SP intake.

The haemoglobin levels of the pregnant women were generally low. As part of the routine antenatal health care, pregnant women are given iron capsules to boost up their haemoglobin levels.

Malaria infection was found to be more prevalent in multigravidae than in primigravidae. Malaria parasite prevalence and parasite density was also higher in the rainy (wet) season than in the dry season.

Blood group O was the predominant blood group among the pregnant women and also provided some amount of protection against malaria. Understanding of the mechanism with which blood group O confers protection against malaria could open doors to further research into the development of drugs or vaccines which recreate the protection provided by this blood type.

Women with higher education give birth to fewer children. The nation's resources can no longer sustain its population because of the

population size. One way the nation can reduce the rate of its population growth, is by encouraging women to pursue higher education.

Recommendations

Authorities should ensure that the antenatal clinics are always well stocked with the SP. In the manufacturing of Drug I, better manufacturing practices should be employed. The appropriate excipients and their correct quantities should be added to the drug. Storage of the drug both at the manufacturing and distribution centres should also be improved.

The giving of free iron capsules to the pregnant women should be sustained by continuously making it available at the clinics.

SP should continuously be administered to the pregnant women at the ANC irrespective of their parities, as they are all susceptible to malaria. Insecticide treated bed nets should be made available at the ANC for free to the pregnant women especially during the wet season to reduce rate of mosquito bites.

The education of girl child should be encouraged. The government could make education of the girl child at all levels of the educational ladder, free with added incentives of free uniforms, free textbooks and free exercise books.

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APPENDICES

APPENDIX 1: Formula for calculating the amount of active ingredient in a tablet;

Milligram = peak area x weight of standard x average weight
of
per tablet   of sample   reference   sample (tablet) in
mg

$$\text{Milligram per tablet in mg} = \frac{\text{peak area of sample} \times \text{weight of standard reference} \times \text{average weight sample (tablet) in}}{\text{average peak area of standard reference} \times \text{weight of sample}}$$

average peak area x weight of sample
of standard reference

Percentage of active ingredient in a tablet

$$= \frac{\text{milligram per tablet} \times 100}{\text{strength of sample (tablet) in milligrams}}$$

=   milligram per tablet   x   100

strength of sample
(tablet) in milligrams

APPENDIX 2: Formula for calculating the amount of active ingredient in a tablet that is dissolved;

Percentage of sample   =   average peak area   x   weight of   x
100
dissolved   of sample   reference

$$\text{Percentage of sample dissolved} = \frac{\text{average peak area of sample} \times \text{weight of reference} \times 100}{\text{average peak area of reference} \times \text{strength of sample (tablet) in grams}}$$

average peak area   x   strength of sample
of reference   (tablet) in grams

APPENDIX 3: How to calculate for desired sample size when population is greater than 10 000;

$n = \dfrac{z^2 \, p \, q}{d^2}$; where

n = the desired sample size (when population is greater than 10 000)

z = the standard normal deviation, usually set at 1.96 (or more simply 2.0),

which corresponds to the 95% confidence level;

p = the proportion in the target population estimated to have a particular

characteristics. If there is no reasonable estimate, then 50% is used;

q = 1.0 − p;

d = degree of accuracy desired, usually set at 0.05 level or occasionally at

0.02.

Therefore, for a population more than 10 000, the desired sample size will be

$n = \dfrac{(2.0^2) \, (0.50) \, (0.50)}{(0.05)^2}$

n = 400

APPENDIX 4: SP STUDY QUESTIONNAIRE FORM

Hospital…………………………….… Date…………………………..

Initial Clinical Presentation

ID number………………………………………………………………………..
Name………………………………………………………………………………
Common name……………………………………………………………………
Folder number…………………………………………………………………….
Age…………………………………………………………………………………

General information

Occupation…………………………………………………………………………
Level of education……………………………………………………………….
Phone number…………………………………………………………………….
Area of residence………………………………………………………………..
House number……………………………………………………………………
Ethnic origin……………………………………………………………………..
Number of pregnancies……………………………….....................................
Number of deliveries…………………………………………………………….
Number of children………………………………………………………………
History of antimalarial taken (Yes or No)……………………………………..
If yes specify and when…………………………………………………………..
Regular sleeping in a mosquito net (Yes or No)………………………………
Frequent use of mosquito coil, insect repellent or insecticide (Yes or No)…….
Recent severe bleeding (Yes or No)……………………………………………..
Blood transfusion (Yes or No)…………………………………………………..
History of dewormer taken (Yes or No)………………………………………
If yes specify and when…………………………………………………………..
History of SP dose taken (Yes or No)…………………………………………
If yes specify (First, Second or Third)…………………………………………
Date of next SP dose……………………………………………………………..
Age of pregnancy…………………………………………………………………
Expected date of delivery………………………………………………………

Results
Blood film species………………………………………………………………..
Parasitaemia………………………………………………………………………
Sickling status (Positive or Negative)………………………………………….
Blood group………………………………………………………………………
Haemoglobin………………………………………………………………………
Strains of *P. falciparum*…………………………………………………………

APPENDIX 5: DRUG ASSAY RESULT SHEETS

Area Percent Report

Page 1 of 1 Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 6-2-2009 2-28-08 PM.dat

Acquired: 6/2/2009 2:28:30 PM   Printed: 10/22/2009 11:38:48 AM

Analyst: GSB

Sample ID: sulfadoxine/pyrimethamine reference   Vial: A01

Injection Volume: 10
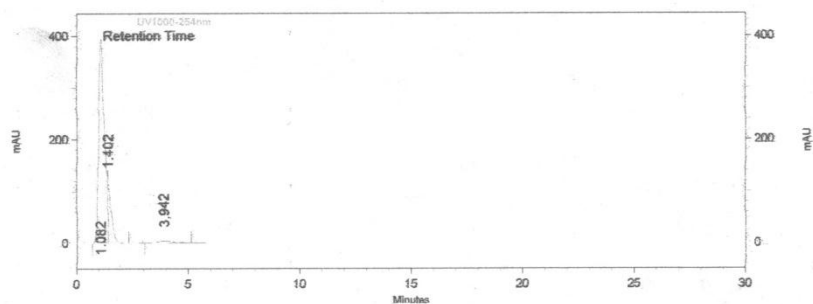
UV1000-254nm
Results (GSB
(6/2/2009
2:35:41 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.082	7112774	83.626	SS
	1.402	1165764	13.706	SS
	3.942	226935	2.668	SS

phenol

Totals				
		8505473	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine – pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq
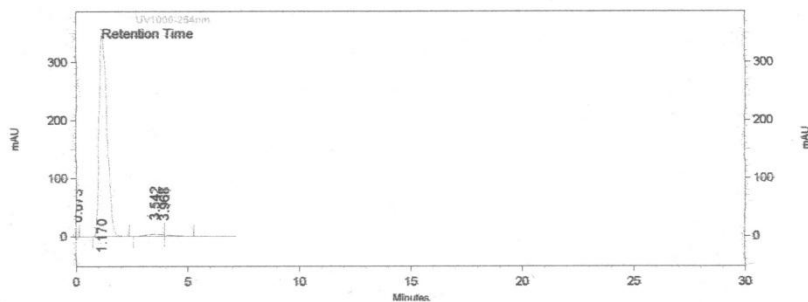
Area Percent Report

Analyst: GSB
Sample ID: sul/pyr ref   Vial: A01   Injection Volume: 10

UV1000-254nm
Results (GSB
(6/11/2009
2:41:04 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	0.073	26494	0.303	BS
	1.170	8479941	97.103	SB
	3.542	163121	1.868	BS
	3.968	63408	0.726	SS
phenol				
Totals		8732964	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 7-20-2009 10-41-44 AM.dat
Acquired: 7/20/2009 10:42:06 AM   **Printed:**
10/22/2009 12:01:39 PM

Analyst: GSB
Sample ID: SUI pyr ref ɒιϛɕo   **Vial:** A01   **Injection Volume:** 10
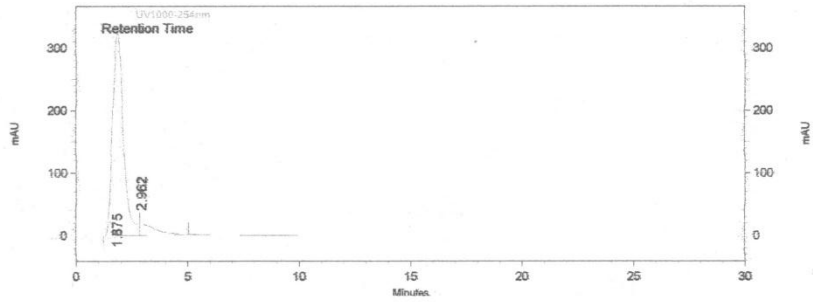
UV1000-254nm
Results (GSB
(7/20/2009
10:55:36 AM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.875	10917895	92.170	SV
	2.962	927539	7.830	VS
phenol				

Totals				
		11845434	100.000	

Instrument Name: Thermo LC1   **Software Version:** Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report
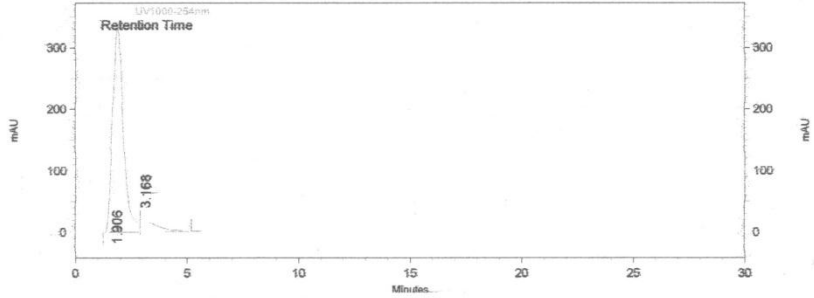
Analyst: GSB
Sample ID: SUl pyr ref ᴅⁱˢˢᴏ   Vial: A01   Injection Volume: 10

UV1000-254nm
Results (GSB
(7/20/2009
11:03:59 AM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.906	11195492	92.038	SV
	3.168	968529	7.962	VS
phenol				

Totals				
		12164021	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

131

Area Percent Report
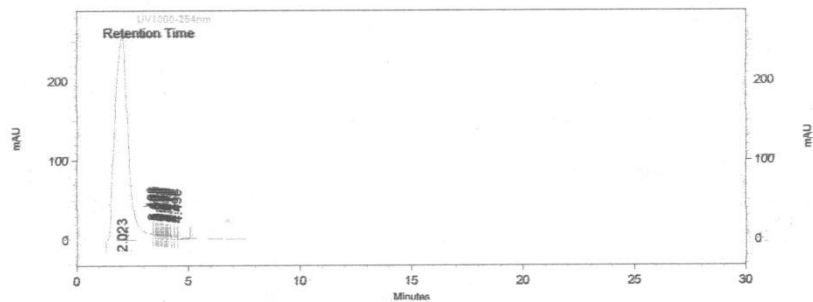
Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 7-20-2009 3-54-40 PM.dat
Acquired: 7/20/2009 3:55:01 PM   Printed: 10/22/2009 12:23:53 PM

Analyst: GSB
Sample ID: fancidar dis ves 2   Vial: A09   Injection Volume: 10

UV1000-254nm
Results (GSB
(7/20/2009
4:03:58 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	2.023	10516241	96.803	BS
	3.408	40268	0.371	SS
	3.508	22993	0.212	SS
	3.568	22152	0.204	SS
	3.628	35051	0.323	SS
	3.728	17738	0.163	SS
	3.782	21236	0.195	SS
	3.895	17653	0.162	SS
	3.955	15022	0.138	SS
	4.008	17891	0.165	SS
	4.075	24939	0.230	SS
	4.228	24009	0.221	SS
	4.342	27587	0.254	SS
	4.495	60818	0.560	SS

phenol

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Acquired: 7/20/2009 11:28:20 AM   Printed:
10/22/2009 12:21:43 PM
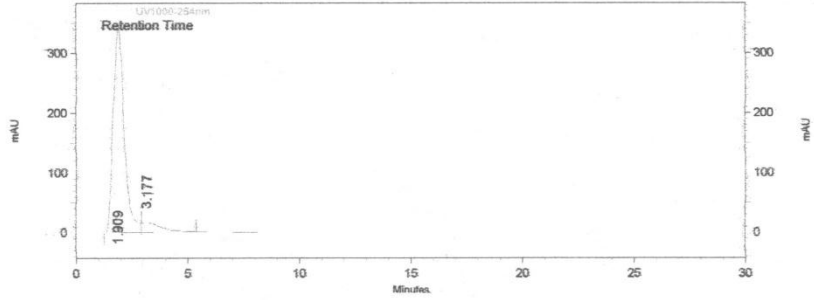
Analyst: GSB
Sample ID: Sul pyr ref disc   Vial: A01   Injection Volume: 10

UV1000-254nm
Results (GSB
(7/20/2009
11:37:41 AM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.909	11725066	92.231	BV
	3.177	987665	7.769	VS
phenol				
Totals		12712731	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 7-20-2009 2-34-13 PM.dat
Acquired: 7/20/2009 2:34:35 PM   Printed:
10/22/2009 12:19:24 PM

Analyst: GSB
Sample ID: palidar dis ves 4   Vial: A06   Injection Volume:
10

UV1000-254nm
Results (GSB
(7/20/2009
2:42:03 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	2.022	7076779	98.403	SS
	3.375	65919	0.917	SS
	3.702	15833	0.220	SS
	3.842	16012	0.223	SS
	3.968	17088	0.238	SS
phenol				
Totals		7191631	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

134

Area Percent Report

Acquired: 7/20/2009 2:34:35 PM   Printed:
10/22/2009 12:19:24 PM

Analyst: GSB
Sample ID: palidar dis ves 4   Vial: A06   Injection Volume:
10

UV1000-254nm
Results (GSB
(7/20/2009
2:42:03 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	2.022	7076779 ·	98.403	SS
	3.375	65919	0.917	SS
	3.702	15833	0.220	SS
	3.842	16012	0.223	SS
	3.968	17088	0.238	SS

phenol

Totals				
		7191631	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 7-20-2009 11-48-57 AM.dat

Acquired: 7/20/2009 11:49:19 AM

Printed: 10/22/2009 12:22:19 PM
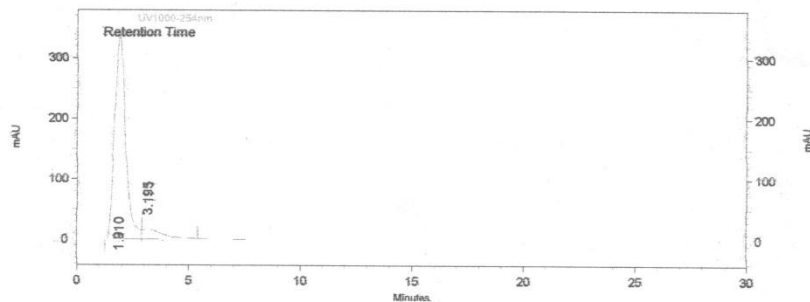
Analyst: GSB

Sample ID: Sul pyr ref diss   Vial: A01   Injection Volume: 10

UV1000-254nm
Results (GSB
(7/20/2009
11:57:54 AM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.910	11588397	91.930	BV
	3.195	1017267	8.070	VS
phenol				

Totals				
		12605664	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 6-11-2009 2-57-04 PM.dat
Acquired: 6/11/2009 2:57:25 PM   Printed:
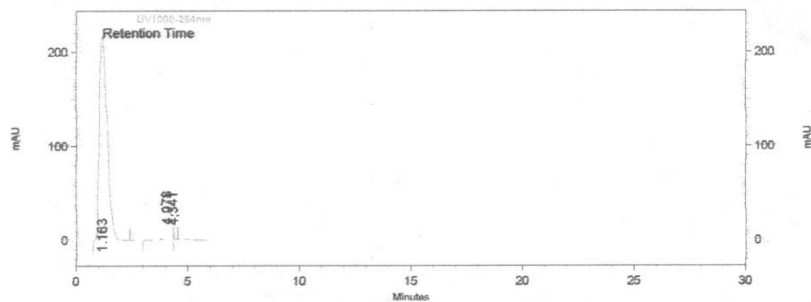10/22/2009 11:55:33 AM

Analyst: GSB
Sample ID: sul/pyr [medriech]   Vial: A02   Injection Volume:
10

UV1000-254nm
Results (GSB
(6/11/2009
3:04:38 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	1.163	5259896	98.355	SS
	4.078	72759	1.361	BS
	4.341	15240	0.285	SS
phenol				

Totals				
		5347895	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
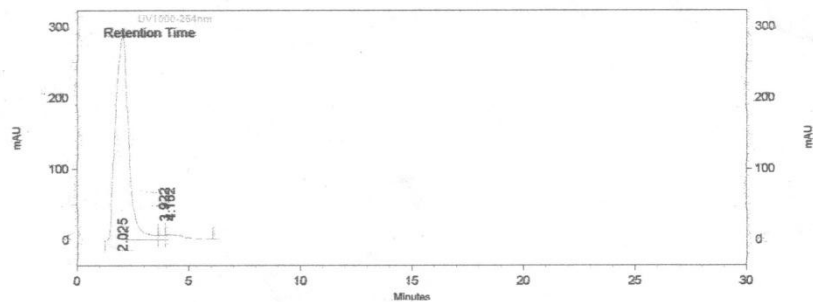Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq

Area Percent Report

Data File: C:\ChromQuest\Projects\Default\Data\drugs\Thermo LC1.10002 7-20-2009 12-47-26 PM.dat
Acquired: 7/20/2009 12:47:47 PM   Printed:
10/22/2009 12:14:40 PM

Analyst: GSB
Sample ID: Medreich dis ves 1   Vial: A02   Injection Volume:
10

UV1000-254nm
Results (GSB
(7/20/2009
12:55:23 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	2.025	11667390	95.904	SV
	3.922	117024	0.962	VS
	4.162	381272	3.134	SB

phenol

Totals				
		12165686	100.000	

Instrument Name: Thermo LC1   Software Version: Version 4.1
Acquisition Method: C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence: C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq
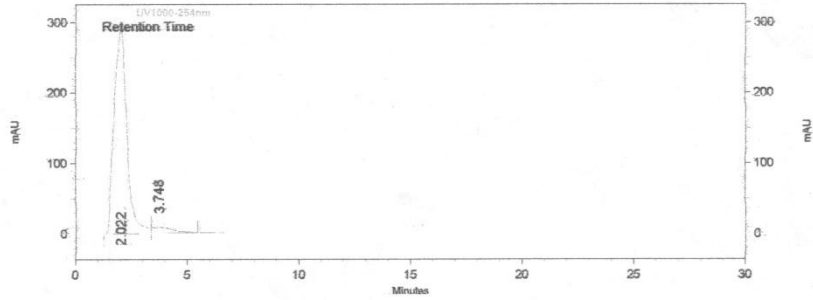
Area Percent Report

Analyst: GSB
Sample ID: Medreich dis ves 1　　　　Vial: A02　　　　Injection Volume: 10

UV1000-254nm
Results (GSB
(7/20/2009
12:46:42 PM)
(Reprocessed)
(Aborted Run))

Name	Retention Time	Area	Area Percent	Integration Codes
	2.022	11564019	95.515	BV
	3.748	543038	4.485	VS
phenol				
Totals		12107057	100.000	

Instrument Name:　　Thermo LC1　　　　Software Version: Version 4.1
Acquisition Method:　C:\ChromQuest\Projects\Default\Method\sulfadoxine - pyrimethamine.met
Sequence:　　　　　C:\ChromQuest\Projects\Default\Sequence\oxytetracycline.seq